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Review article

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Crimean–Congo haemorrhagic fever virus

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Abstract

Crimean–Congo haemorrhagic fever (CCHF) is a severe tick-borne illness with a wide geographical distribution and case fatality rates of 30% or higher. Caused by infection with the CCHF virus (CCHFV), cases are reported throughout Africa, the Middle East, Asia and southern and eastern Europe. The expanding range of the *Hyalomma* tick vector is placing new populations at risk for CCHF, and no licensed vaccines or specific antivirals exist to treat CCHF. Furthermore, despite cases of CCHF being reported annually, the host and viral determinants of CCHFV pathogenesis are poorly understood. CCHFV can productively infect a multitude of animal species, yet only humans develop a severe illness. Within human populations, subclinical infections are underappreciated and may represent a substantial proportion of clinical outcomes. Compared with other members of the *Bunyavirales* order, CCHFV has a more complex genomic organization, with many viral proteins having unclear functions in viral pathogenesis. In recent years, improved animal models have led to increased insights into CCHFV pathogenesis, and several antivirals and vaccines for CCHFV have shown robust efficacy in preclinical models. Translation of these insights and candidate therapeutics to the clinic will hopefully reduce the morbidity and mortality caused by CCHFV.

Sections

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Introduction

Crimean–Congo haemorrhagic fever virus (CCHFV) was first reported in the 1960s as a cause of febrile illnesses in the Congo[1]. Investigation of febrile illnesses in Crimea showed that the virus responsible for these cases was antigenically identical to the cause of illnesses in the Congo[2]. Since then, serological studies and reported human cases demonstrate that CCHFV is a widely distributed haemorrhagic fever virus endemic throughout Africa, the Middle East, Southeast Asia and southern and eastern Europe (reviewed in ref. [3]), closely following the range of its *Hyalomma* tick reservoir host. Studies have identified ticks of the *Hyalomma* genus to be the principal vector and reservoir of CCHFV, although other tick species may have a role in maintaining CCHFV in endemic regions (reviewed in ref. [4]). Long-range transport of CCHFV-infected ticks on birds[5], global trade leading to the introduction of tick vectors to new continents[6] and climate change leading to an expanding range of the *Hyalomma* tick as far north as Sweden[7] suggest that the geographical range of CCHFV will continue to expand. Serological studies have shown that CCHFV can productively infect diverse wild animal species such as hares, small rodents, ostriches, buffalo and even rhinoceroses[8] and, importantly for human exposure, livestock without apparent disease (reviewed in ref. [8]). These animal species serve as important amplifying hosts for CCHFV, enabling CCHFV to spread from infected ticks to uninfected ticks through either co-feeding or feeding on a viraemic animal[9]. Humans are most often infected with CCHFV through tick bites or handling and butchering of infected livestock. On infection, CCHF begins as a nonspecific febrile illness that can then progress to the severe haemorrhagic manifestations. Case fatality rates vary but can be higher than 30% in some regions. There are currently no approved vaccines or therapeutics for CCHF. In recent years, we have gained an improved understanding of the function of CCHFV proteins in viral replication, and improved animal models have provided important insight into CCHFV pathogenesis and enabled preclinical testing of multiple vaccine platforms and therapeutic strategies for CCHF. In this Review, we focus on recent insights gained into the function of viral proteins in CCHFV pathogenesis along with our current understanding of CCHF and the state of treatments and vaccines for CCHFV.

Molecular biology of CCHFV

CCHFV is an enveloped negative-sense RNA virus belonging to the Orthonairovirus genus in the *Nairoviridae* family of the *Bunyavirales* order[10] (Fig. 1a). In addition to CCHFV, the *Nairoviridae* family consists of arthropod-borne viruses such as Nairobi sheep disease virus, Dugbe virus and Hazara virus (HAZV), although these viruses seem to cause little-to-no disease in humans[11]. As in other bunyaviruses, the tri-segmented viral genome is coated with the viral nucleoprotein (NP) and bound by the L protein[12] (Fig. 1a). The viral proteins are encoded by three genomic segments (Fig. 1b), and relative to other members of the order that can cause human disease, CCHFV has a more complex genomic organization (discussed below). On entry, these proteins produce positive-sense viral RNA using the genomic negative-sense viral RNA as a template to initiate viral protein production and replication[12] (Fig. 1c). The viral glycoproteins Gn and Gc are found on the virion surface and are responsible for receptor binding and viral entry (Fig. 1a).

S segment

The small genomic segment (S) encodes the viral NP along with the small non-structural protein (NSs) in an opposite-sense reading frame (Fig. 1b). In addition to interactions with the viral RNA to form ribonucleoprotein complexes, NP possesses endonuclease activity, interacts with host heat shock proteins during intracellular viral replication and in infectious particles, and promotes translation of viral mRNAs[13–16], demonstrating a multifactorial role for NP in the CCHFV life cycle. NP and NSs may also modulate host cell apoptosis[13,17–19], suggesting that regulation of host cell apoptosis is important for the CCHFV life cycle. CCHFV infection induces host cell apoptosis in vitro and in vivo[18,20,21], and biomarkers of apoptosis were found to be upregulated in patients infected with CCHFV[22,23]. However, it is unclear whether host apoptosis is proviral or antiviral. Inhibition of host apoptosis in vitro resulted in increased viral titres suggestive of an antiviral effect[17]. The CCHFV NP can suppress activation of caspase 3 and caspase 9, and induction of apoptosis triggered by BAX and the release of cytochrome *c* from mitochondria, although it is unclear where in the intrinsic apoptosis pathway NP blocks activation[18] (Fig. 1c). Together, these data indicate that host apoptosis may exert antiviral activity against CCHFV and that CCHFV utilizes its NP protein to suppress this host response. Yet, the CCHFV NSs can disrupt mitochondrial membrane potential, thus triggering apoptosis[19], and later during infection, CCHFV induces activation of BID (a pro-apoptotic protein), probably through extrinsic apoptotic signals[18], suggesting that CCHFV may also promote apoptosis (Fig. 1c). Furthermore, the CCHFV NP and related HAZV NP contain a highly conserved DEVD or DQVD cleavage motif, respectively, that is cleaved by host caspase 3 (refs. [13,17,24]) (Fig. 1c). Although it has been suggested that cleavage of NP by caspase 3 may be a host defence against CCHFV[17], structural studies have shown that oligomeric conformations of NP result in shielding of this motif from host caspase 3 (ref. [25]). Thus, NP may be cleaved by host caspase 3 only when present in specific conformations[25]. Mutation of viral NP to eliminate caspase 3 cleavage resulted in enhanced viral RNA polymerase activity[25], suggesting that cleavage of NP may regulate viral RNA synthesis. However, infectious HAZV and CCHFV could be rescued when the cleavable motifs were replaced with an uncleavable DQVE or AEVA motif, respectively[24,26], and a CCHFV mini-replicon system showed equivalent reporter activity between wild type NP and NP with an altered DEVD motif[13], demonstrating that host caspase cleavage of this motif in orthonairoviruses is not essential to viral replication. Interestingly, infectious HAZV possessing a similarly uncleavable AQVA motif could not be rescued, suggesting that this motif may have important functions distinct from caspase cleavage[24]. The high conservation of the DEVD motif in CCHFV further suggests that any host antiviral activity exerted through caspase 3 cleavage of NP is offset by yet-unclear proviral functions. The role of host cell apoptosis and caspases in CCHFV infection may be distinct in mammalian and tick hosts. HAZV growth in tick cells did not induce apoptosis nor was HAZV NP cleaved by tick caspases[27], yet the CCHFV possessing the uncleavable AEVA motif failed to grow in tick cell culture[26].

M segment

The CCHFV medium (M) segment encodes the viral glycoprotein precursor (GPC). Compared with the M segment of other *Bunyavirales*, the CCHFV M segment is complex. The GPC is proteolytically processed to produce the individual viral glycoproteins Gn and Gc[28], a GP160/85 protein that is further proteolytically processed[29] to a heavily glycosylated mucin-like domain (MLD)[28] and a GP38 protein[29], and a medium non-structural protein (NSm) that promotes glycoprotein processing and virion assembly[30] (Fig. 1b). Proteolytic processing of the GPC occurs through host furin-like and SKI-1 proteases as the proteins traffic through the endoplasmic reticulum and Golgi apparatus[29]. Among the *Bunyavirales* order, Gn and Gc participate in receptor binding and entry

(reviewed in ref. [31]), and CCHFV undergoes clathrin-dependent and pH-dependent fusion and entry[32]. However, the roles of the other GPC-encoded proteins are less clear. Localization signals to direct nascent proteins to the Golgi apparatus seem to be localized to Gn, although MLD and GP38 may also participate in directing proper processing of the GPC[33,34]. The GP160/85 protein is released from the pre-Gn protein in the Golgi apparatus by host proteases[35] and is found in supernatants of infected cells[29], but whether it has a biological function in this context is unknown. The MLD protein is heavily glycosylated and has little sequence conservation across diverse CCHFV isolates[36,37], suggesting it is under diversifying selective pressure; yet, its role in the CCHFV life cycle is unclear. Deletion of the gene encoding the MLD protein did not impair infectivity of virus-like particles (VLPs) but did lead to a reduction in incorporation of the CCHFV glycoproteins into particles[30]. Ebola virus (EBOV) possesses an MLD that contributes to EBOV-induced endothelial membrane disruption and vascular permeability[38] along with downregulation of major histocompatibility complex (MHC)-I on the surface of infected cells to block T cell activation[39]. Whether the CCHFV MLD performs similar functions is unknown. GP38 promotes proper virion assembly[30] and may be secreted alone or linked to the MLD as part of the GP160/85 protein[28,29]. Limited evidence from the study of VLPs suggests that GP38 may be found on the viral envelope and plasma membrane[40]; however, other studies have failed to detect GP38 in authentic CCHFV virions[29] (Fig. 1a). Nevertheless, its function in these contexts is unknown.

NSm seems non-essential for CCHFV infection of mammalian hosts as recombinant CCHFV lacking NSm was able to replicate and cause lethal disease in *Ifnar*$^{-/-}$ mice[41]. However, it is possible that NSm retains functions in the tick or interferon (IFN)-competent host, as adaptation of CCHFV to either resulted in mutations in the NSm protein[42,43]. Nevertheless, the function of the NSm in these hosts is unknown.

L segment

Consisting of more than 12,000 nt, the large (L) segments of CCHFV and related orthonairoviruses are unusually large for members of the *Bunyavirales* order[44] (Fig. 1b). The encoded L protein contains the viral RNA-dependent RNA polymerase[44] and cap-snatching functions[45]. Interestingly, at the very amino terminus, the Orthonairovirus L protein contains an ovarian-tumour-like (OTU) protease that possesses both de-ISGylating and deubiquitylating functions[46]. RIG-I has been shown to initiate the type I interferon response to CCHFV[47], and the OTU domain antagonizes RIG-I-mediated host innate immunity through its deubiquitylation function[48] (Fig. 1c). The de-ISGylating function may also modulate host immunity as ISG15 modifications of viral proteins and the ISG15 protein itself can be antiviral[49]. The OTU domain may regulate viral replication beyond antagonizing innate immunity[48,50]. Although some data suggest that the OTU domain may regulate the viral RNA polymerase through host ISG15 (ref. [51]), other data suggest that the defect in OTU-inactive viruses is due to stable occupancy of the OTU catalytic domain by ubiquitin, resulting in impaired RNA polymerase activity[48]. Species-specific preferences in the OTU domains of CCHFV and related viruses for either ubiquitin or ISG15 have been hypothesized to mediate host susceptibility to disease[52,53]. Curiously, across the *Bunyavirales* order, human pathogenic viruses typically possess antagonists of innate immunity in their NSs and NSm proteins (reviewed in ref. [54]). Yet, similar functions have not been shown for the CCHFV NSs or NSm proteins[54], and the OTU domain is the only identified direct antagonist of the type I interferon response to CCHFV.

Genetic diversity

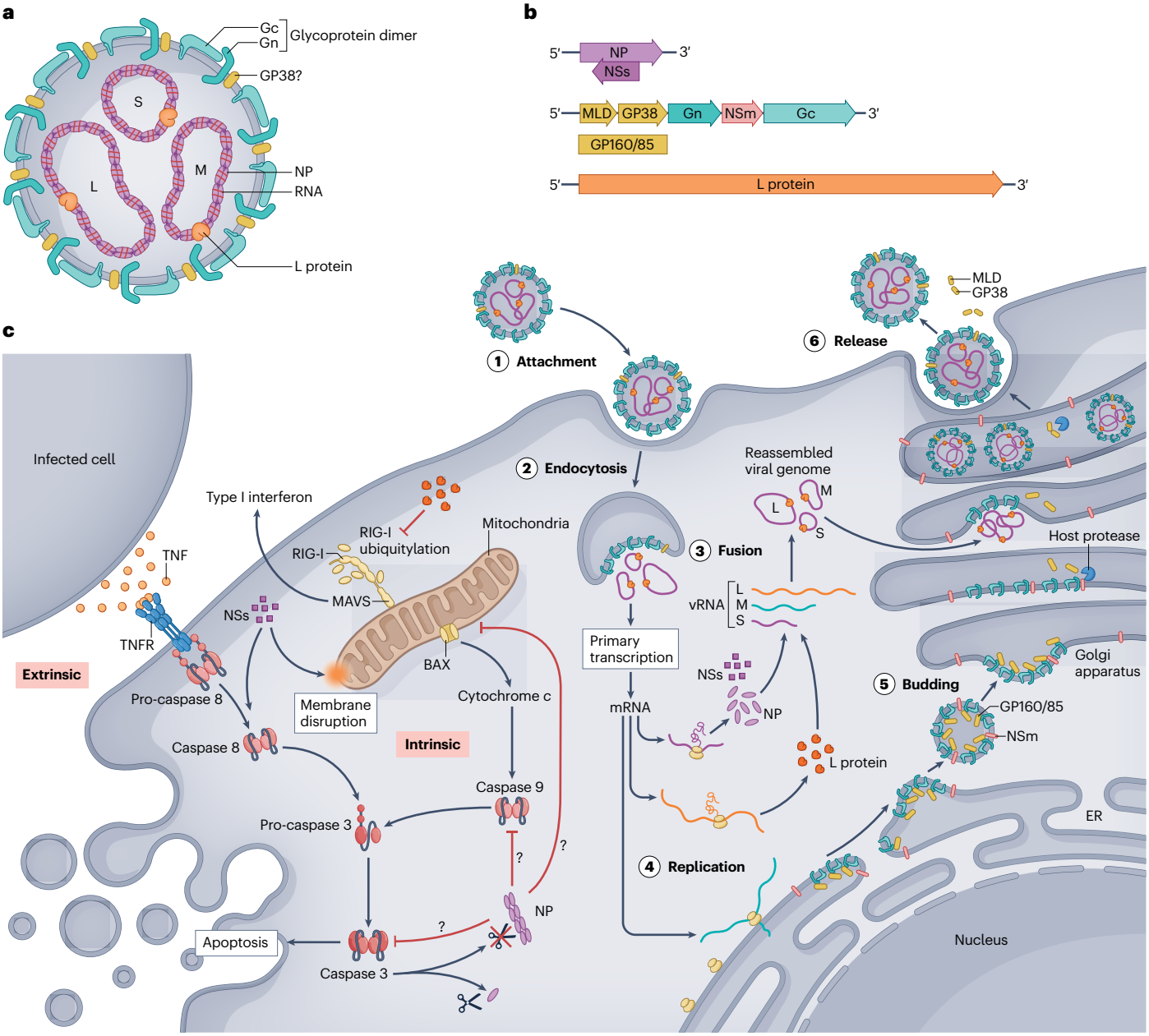
Corresponding with its wide geographical distribution, CCHFV is a genetically diverse virus. Although the NP and L proteins of CCHFV strains are conserved with approximately 95% or more amino acids conserved between strains, the CCHFV GPC is much less conserved, with divergent strains exhibiting less than 75% amino acid conservation[36,37]. The genetic diversity of CCHFV correlates strongly with geography, and clades of CCHFV segregate based on geographical location[37,55]. It is unknown what selective pressures drive the sequence diversity of CCHFV across its geographical range. Given that humans are incidental hosts for CCHFV, the selective pressures acting on CCHFV probably arise in the tick reservoir or mammalian amplifying hosts. Interestingly, CCHFV strains isolated from similar regions decades apart show strong sequence conservation[56,57], suggesting that temporal evolution of CCHFV within geographical regions is limited. Instead, genetic diversity may arise from long-range migration. Strains of CCHFV circulating in southwestern Europe cluster with African rather than eastern European isolates[58], indicating the long-range introduction of CCHFV to Europe from Africa, potentially through migratory birds carrying CCHFV-infected ticks[5]. In addition, the segmented genome of CCHFV can undergo reassortment, and CCHFV isolates possessing genomic segments with distinct geographical lineages have been used to identify historical migration and co-circulation of CCHFV over long geographical distances[55,59].

Transmission to humans and risk factors

The endemicity of CCHFV is closely associated with the geographical distribution of the main arthropod vector and reservoir, *Hyalomma* ticks[4]. As an arthropod-borne virus, CCHFV is transmitted to humans primarily through the bite or handling of an infected tick, and in endemic regions, tick bites may be common and not recognized as a risk factor for CCHFV infection. CCHFV, however, can also be transmitted to humans by direct contact with blood or tissues of viraemic animals, mainly livestock. Thus, high-risk exposure exists for people with outdoor activities (for example, soldiers, farmers, forest workers and hikers) and those with close contact to livestock (for example, shepherds, farmers, butchers, slaughterhouse workers and veterinarians)[56,60–64]. In addition, surges in CCHFV may be seen during religious festivals such as Eid-ul-Adha in which potentially CCHFV-infected livestock are transported from rural to urban areas for slaughter by potentially untrained individuals[65,66]. Nosocomial and intrafamily transmission have been reported through needlesticks or contact with blood and secretions from patients, putting health-care workers and close family members involved inpatient care at risk for exposure[64,67,68]. Transmission of CCHFV during aerosol-generating medical procedures[69] or sexual contact[70] may be possible. However, CCHFV transmission from human to human seems to be inefficient, and widespread outbreaks sustained via human-to-human transmission (Box 1), such as those that have occurred multiple times with EBOV (reviewed in ref. [71]), have not been reported for CCHFV.

Crimean–Congo haemorrhagic fever

Serological evidence demonstrates that CCHFV can productively infect a wide variety of domestic and wild animal species from rabbits to cattle to ostriches to tortoises[8,72], yet only humans develop symptomatic disease. In humans, CCHF can display various outcomes ranging from asymptomatic and mild infections to severe and sometimes lethal disease[64,73]. Clinical diagnosis of CCHF is difficult as initial symptoms are similar to those of other febrile illnesses (Box 1). Therefore, laboratory testing has a pivotal role in case management and outbreak control (Box 2).



CCHF presents in four distinct stages: incubation, pre-haemorrhagic, haemorrhagic and convalescence[64,74–76] (Fig. 2). The incubation period is usually less than a week (range 1–9 days) and depends on the route of exposure and virus dose. It seems shortest following a tick bite (usually 1–3 days) and slightly longer following exposure to blood, tissue and secretions of infected livestock and humans (5–6 days). The pre-haemorrhagic stage lasts about 2–4 days on average (range 1–7 days) and begins abruptly with rather nonspecific symptoms including fever (39–41 °C), headache, myalgia, dizziness, neck pain and stiffness, backache, headache, sore eyes and photophobia[74]. This may be accompanied by sore throat, abdominal pain, nausea, vomiting and diarrhoea[63,74]. Hyperaemia of the face, neck and chest, congested sclera and conjunctivitis, and jaundice may also be noticed[64]. In severe cases, changes in mood and sensory perception have been reported. Somnolence may replace agitation[77,78]. Hepatomegaly and splenomegaly may also be present[63].

The haemorrhagic stage is usually short (approximately 2–3 days) but can be prolonged up to 2 weeks[74]. Haemorrhagic manifestations range from petechia to extended ecchymoses on mucous membranes and skin, a finding particularly pronounced with CCHF compared with other viral haemorrhagic fevers (VHFs). Epistaxis, melena, haematemesis, haematuria and haemoptysis are common as is bleeding from injection sites[74,77]. Bleeding has occasionally been reported from other sites such as the vagina, uterus and brain[79–81]. Haematology and blood chemistry commonly show thrombocytopenia, leukopenia and elevated levels of aspartate aminotransferase (AST), alanine aminotransferase

Fig. 1 | Molecular biology of Crimean–Congo haemorrhagic fever virus. **a**, Crimean–Congo haemorrhagic fever virus (CCHFV) is an enveloped, tri-segmented, negative-sense RNA virus. The virion is studded with the glycoproteins Gn and Gc, which mediate receptor binding and entry. Some evidence suggests that the GP38 accessory protein is also found on the virion but if so, with unclear localization or function. **b**, The three genomic segments of CCHFV are the small (S), medium (M) and large (L) segments. The S segment encodes the viral nucleoprotein (NP) in one reading frame and the small non-structural protein (NSs) in an opposite-sense open reading frame. The M segment is complex, encoding a glycoprotein precursor (GPC) that is processed by host proteases to produce a GP160/85 domain that is further processed to a mucin-like domain (MLD) and GP38, the Gn and Gc glycoproteins and the medium non-structural protein (NSm). The L segment of CCHFV is unusually large for bunyaviruses, and the encoded protein contains the viral RNA-dependent RNA polymerase (RdRP) and an ovarian tumour-like protease (OTU) at the N terminus. **c**, On attachment (stage 1), CCHFV undergoes clathrin-dependent and pH-dependent entry into the host cytoplasm (stages 2 and 3). After entry into the cytoplasm, viral genomes are converted to positive-sense mRNA by the RdRP and initiate translation of viral proteins. These proteins also coordinate to produce new negative-sense viral genomes that are coated with NP and a bound L protein to initiate replication on infection of the next cell (stage 4). The GPC is translated into the endoplasmic reticulum (ER) and during trafficking through the ER and Golgi apparatus is proteolytically processed to produce mature glycoproteins along with the accessory proteins MLD, NSm and GP38. Newly produced genomes are packaged into enveloped particles and the virus buds into the Golgi apparatus for release via the secretory pathway (stage 5). New virions are then released to infect additional cells, whereas GP160/85, MLD and GP38 are also released extracellularly but with unclear consequence (stage 6). In addition to facilitating viral replication, CCHFV proteins also block host apoptosis and innate immune pathways. The CCHFV NP can block the intrinsic pathway of apoptosis at a yet-to-be-defined step, whereas the CCHFV NSs promotes apoptosis through disruption of the mitochondrial membrane or extrinsic apoptotic pathways. CCHFV may also promote apoptosis through production of tumour necrosis factor (TNF) and the TNF death receptor pathway. The CCHFV NP is also cleaved by host caspase 3 but oligomeric conformations may block this cleavage. The OTU domain of the CCHFV L protein blocks RIG-I-dependent initiation of the type I interferon response via its deubiquitylating function. MAVS, mitochondrial antiviral signalling protein; vRNA, viral RNA.

(ALT), lactate dehydrogenase and creatine phosphokinase[74,78,82–84] along with elevated levels of inflammatory cytokines[85,86]. Coagulation may be affected, with prolonged prothrombin and activated partial thromboplastin times accompanied by a decrease in fibrinogen levels and an increase in the levels of fibrinogen degradation products[74,78,82,84]. The haemorrhagic stage is pronounced in severe cases, with rapid progression to disseminated intravascular coagulation, overt bleeding, kidney, liver or pulmonary failure, and shock[74,87–89]. If lethal, death usually occurs in the second week of illness.

In survivors, convalescence generally begins around 9–10 days post-onset of illness (range 9–20 days) and is associated with a return to normal for laboratory parameters[74,75,90]. This stage can be prolonged and may be associated with hypotension, tachycardia or bradycardia, polyneuritis, breathing issues, xerostomia, vision and hearing deficiencies, hair loss and memory loss among others[73]. There is no reliable evidence for relapse or a biphasic course of the disease; however, sequelae have not been studied well enough to determine long-term complications. Survivors typically develop humoral and cellular immunity against CCHFV[91,92].

Correlates of disease outcome

Viraemia has prognostic significance for the outcome of a CCHFV infection. Patients with titres exceeding 10^9 genome copies per millilitre of plasma are more likely to have lethal disease, and mean values for fatal cases are >1,000-fold higher than those of patients who survive[86,93,94]. Along with high viraemia, early clinical laboratory criteria (up to 5 days after onset) that may predict fatal outcome are thrombocytopenia (≤150,000 platelets per microlitre), elevated AST and ALT levels and elevated levels of pro-inflammatory cytokines[74,77,83–85,87,95]. In addition, overt disseminated intravascular coagulation, haematemesis, melena and somnolence were associated with fatal outcomes[77,84]. Scoring systems that comprehensively evaluate patients for multiple risk factors have been developed and are accurate predictors of death[77,84]. Early host antibody responses may also be a predictor of disease outcome. Antibody responses to CCHFV may be rapid, with CCHFV-specific IgM detectable as soon as 2–3 days after symptom onset and CCHFV-specific IgG within 5–6 days[96–98]. Clearance of viraemia correlates with early IgM responses[96], whereas in fatal cases, there is little evidence of an antibody response against CCHFV[91,97], suggesting that a failure to mount anti-CCHFV humoral immunity may result in lethal outcomes.

The contributions of viral determinants to disease severity are unknown and it is unclear whether the genetic diversity of CCHFV contributes to the varied case fatality rates reported throughout endemic regions. A highly divergent lineage of CCHFV first recognized in Greece[99] may account for high seroprevalence without associated clinical cases in this region, suggestive of reduced human pathogenic potential[100,101]. Disease severity is probably also a function of route of exposure, amount of inoculating virus and level of public health resources available to treat CCHFV infections. Host genetics may also contribute to disease outcome[102–107]. Subclinical infections with CCHFV are probably widely underappreciated, and better recognition of milder disease cases may alter case fatality rates[100,108].

Insights into CCHF from animal models

Rodent models. Although initially used mainly for viral propagation, suckling mice exhibit lethal disease on infection with CCHFV and have been used to evaluate efficacy of antivirals and monoclonal antibodies[33,109]. More recently, adult mice deficient in type I interferon through genetic deficiency in the IFNα receptor (*Ifnar*$^{-/-}$), transient suppression of IFNAR signalling or genetic deficiency in signal transducer and activator of transcription 1 (*Stat1*$^{-/-}$) have been used as models of lethal CCHFV[21,110,111]. Hamsters deficient in STAT2 are also susceptible to lethal CCHFV infection[112]. In these models, disease is typically associated with uncontrolled viral replication, inflammatory immune responses, liver pathology and eventually death[21,110–113]. Lethal disease in these rodent models has similar correlates to lethal disease in humans, suggesting that similar disease mechanisms may result in lethal outcomes in infected mice, hamsters and human patients. These models have been valuable tools for preclinical evaluation of antivirals[112,114–116], monoclonal antibodies[40,117], vaccines[118] and host-directed therapies[119].

Mouse models have also provided valuable insight into CCHFV pathogenesis. In mice, hepatocytes and endothelial cells are targets of CCHFV infection[21,110,114,120], and viral replication in these tissues may account for the liver damage and vascular dysfunction seen in CCHF cases. Infection of monocytes by CCHFV may also contribute to disease progression[21,120,121]. Infection of innate immune cells such

Box 1

Crimean–Congo haemorrhagic fever virus and other viral haemorrhagic fevers

Viral haemorrhagic fever (VHF) is a clinical syndrome that includes fever and different degrees of haemorrhaging. VHFs may range from mild to lethal disease depending on pathogen and host factors further influenced by case patient management. Crimean–Congo haemorrhagic fever virus (CCHFV) is one of many RNA viruses from distinct virus families that may cause VHFs: *Arenaviridae* (Lassa fever, Lujo haemorrhagic fever (HF), Chapare HF, Junin HF, Machupo HF and Sabia HF), *Filoviridae* (Ebola virus (EBOV) disease and Marburg virus disease), *Flaviviridae* (Alkhurma HF, Dengue fever, Kyasanur Forest disease, Omsk HF and yellow fever), *Hantaviridae* (HF with renal syndrome and hantavirus pulmonary syndrome) and *Phenuiviridae* (Rift Valley fever). VHFs are often difficult to distinguish by clinical presentation alone. Initial symptoms are nonspecific including fever, fatigue, dizziness, myalgia, weakness and exhaustion that can be accompanied by gastrointestinal and neurological symptoms. More severe cases may develop coagulation abnormalities that may progress to disseminated intravascular coagulation, single or multiple organ failure, shock, coma and death. VHFs should be considered life-threatening diseases and patients should be isolated because of the potential of human-to-human transmission. VHFs are zoonotic diseases harboured in certain reservoir animal species, thus dictating their geographical distribution; some of them are arthropod-borne and transmitted by certain ticks or mosquitoes. Following the initial zoonotic introduction into humans, some VHFs are associated with varying ability of human-to-human transmission and have the potential to cause epidemics such as the 2014–2016 EBOV outbreak in West Africa. Others such as yellow fever virus can cause outbreaks sustained through human-to-mosquito-to-human transmission cycles. Thus, knowledge about the ecology and epidemiology of VHFs together with a detailed case and travel history may provide valuable insight into distinguishing and diagnosing VHFs. Ultimately, the diagnosis can only be achieved by laboratory testing, which often requires the involvement of national or regional reference laboratories owing to limited testing availability and biocontainment classification of certain VHF-causing viruses. Rapid diagnosis is important for proper case patient management and public health measures. Case patient management in general is based on supportive therapy targeting life-threatening symptoms. Currently available antivirals such as ribavirin may be helpful for the treatment of some VHFs if started early in disease progression. More antivirals, such as nucleoside analogues, polymerase inhibitors and monoclonal antibodies, are in preclinical stages of development for several VHFs. Later in disease, certain immunosuppressive therapies may be indicated to target syndromes such as a cytokine storm. Vaccines are under development for many VHF-causing viruses but only a few vaccines are licensed, such as Ervebo (Merck) for EBOV. Control of reservoir species can be difficult when it comes to rodents and ticks. Overall, more attention has been given to VHFs lately as emerging or re-emerging infectious diseases with epidemic potential. Achievements in VHF diagnosis are obvious but implementation of testing in endemic areas remains challenging. More basic and translational research is needed for development of urgently needed countermeasures.

as monocytes and macrophages by EBOV leads to a dysregulated and overwhelming inflammatory response (reviewed in ref. [122]), a central feature of several VHFs[123]. Type I interferon-deficient mice infected with CCHFV exhibit many features of a dysregulated inflammatory immune response[110,119,120], suggesting that mechanisms of immunopathogenesis in CCHF are similar to those seen in other VHFs. Transient suppression of type I interferon signalling through administration of the IFNAR-blocking antibody MAR1-5A3 has been used to investigate CCHFV infection in knockout mice to evaluate the host contribution to disease. Using this transient suppression, CCHFV-infected mice deficient in adaptive immunity (*Rag2*$^{-/-}$) or perforin showed severe liver pathology, demonstrating that liver damage occurred in the absence of cytotoxic T cells[21]. Instead, activation of the tumour necrosis factor (TNF) superfamily and extensive apoptosis in infected livers suggest that liver pathology may occur through induction of apoptosis in infected and uninfected bystander cells[21]. Further studies showed that CCHFV infection of mice deficient in mitochondrial antiviral signalling protein (MAVS) and treated with the IFNAR-blocking antibody were protected from disease, suggesting that in the absence of type I interferon, MAVS signalling may contribute to poor outcomes[119]. In addition, in this model, blockade of TNF through neutralizing antibody treatment could protect against death[119]. Together, these data suggest that host inflammatory responses contribute to morbidity and mortality on infection with CCHFV. In a mouse model recapitulating the convalescent phase of disease[120], depletion of $CD4^+$ and/or $CD8^+$ T cells or blockade of IFNγ signalling resulted in decreased survival, suggesting that cellular immunity and type II interferon exert control on CCHFV infection[124]. Furthermore, humanized mice (NSG-SGM3) engrafted with human stem cells developed CCHFV strain-specific disease outcomes ranging from mild, self-limiting disease to progressive lethal disease[125]. Fatal disease was associated with viral replication in glial cells and severe neurological disease[125]. This model may be useful for studying the potential neurological involvement of CCHFV.

The conversion of an asymptomatic infection with CCHFV in immunocompetent mice to a rapidly lethal infection in type I interferon-deficient mice demonstrates that type I interferon is a key restriction factor for CCHFV in the mammalian host. How the host senses CCHFV and initiates the innate immune response in vivo is unclear. Examining how innate immunity controls CCHFV and how CCHFV antagonizes innate immunity to cause disease in humans is difficult in type I interferon-deficient models. Furthermore, although type I interferon deficiency results in profound defects in innate immunity,

this deficiency probably also extends to adaptive immunity (reviewed in refs. [126,127]), confounding studies of both innate and adaptive immunity to CCHFV. Recently, a mouse-adapted strain of CCHFV was isolated that is able to cause disease in immunocompetent wild type laboratory strains of mice[43]. Infection of mice with this mouse-adapted strain of CCHFV resulted in inflammatory cytokine production, high viral loads in multiple tissues, pathology in the liver and spleen, and convalescence was associated with robust humoral and cellular immunity[43]. Studies in *Ifnar*$^{-/-}$ and *Rag1*$^{-/-}$ mice demonstrated that both type I interferon and adaptive immune responses exert control of CCHFV in this model[43], enabling investigations into how host immune responses control CCHFV. Sequencing of this virus identified five coding mutations: two in the viral NP with one also mutating the viral NSs, one in the NSm and two in the L protein[43]. Accumulation of mutations in these viral proteins suggests that these proteins are involved in CCHFV pathogenesis in immunocompetent hosts. Unexpectedly, a sex-linked bias in disease severity was observed, with female mice largely resistant to severe disease. The more severe disease observed in male mice was associated with similar correlates of poor outcome in human CCHF cases such as greater inflammatory cytokine production, prolonged viraemia and greater tissue pathology[43]. Although sex-linked differences have been occasionally reported for CCHFV[128–131], further studies that account for cultural practices that place men at greater risk for exposure to CCHFV[61] will be needed to determine whether similar sex-linked differences in disease outcome are present in infected humans.

Non-human primate models. In addition to rodent models, non-human primate (NHP) models of CCHF have been developed. Cynomolgus macaques infected with CCHFV recapitulate many aspects of human CCHFV, including varied disease outcome. In an initial report, infection of cynomolgus macaques with a human clinical isolate, CCHFV strain Hoti, resulted in severe disease in all animals infected via the intravenous or combined intravenous and subcutaneous routes[132]. Four of eight animals across these groups were euthanized by day seven post-infection because they had reached humane end point criteria[132]. Severe disease was associated with liver pathology, inflammatory cytokines, high viral loads and coagulation disorders, similar hallmarks to severe human disease[132].

However, continued reports on the model have demonstrated more variable disease outcomes. In studies evaluating the antiviral favipiravir in CCHFV-infected macaques, only one of eight animals in the placebo group reached euthanasia criteria by day eight and disease was generally moderate in the remaining placebo-treated animals[133]. Similarly, cynomolgus macaques infected with strain Hoti or another human clinical isolate strain, Afghan-09, developed mild-to-moderate disease characterized mainly by transient viraemia and thrombocytopenia, and no animals reached euthanasia criteria[134].

Box 2

Crimean–Congo haemorrhagic fever diagnostics

Crimean–Congo haemorrhagic fever (CCHF) may initially present like other febrile illnesses, and rapid and accurate diagnosis is critical for both case management and prevention of transmission. Patient history such as living in or visiting an endemic area, profession, outdoor activities, history of tick bite or contact with a known case can provide important insight, but a final diagnosis requires laboratory testing. CCHF virus (CCHFV) laboratory diagnostics are accomplished directly through detection of the virus or indirectly through determining the host immune response to infection. The preferred clinical specimen is whole blood or serum/plasma. Owing to biosafety concerns, precautions should be taken when handling diagnostic specimens before inactivation. Consecutive samples from the same patient may help to address some sensitivity issues.

The method of choice for rapid CCHFV detection is quantitative reverse transcription PCR (qRT–PCR). A few commercially available assays are designed to detect the broader range of CCHFV lineages. The tremendous genetic variability of CCHFV through virus mutations and genomic segment reassortment or recombination, however, may require the use of CCHF lineage-specific assays to address geographical alterations[177]. Rural settings may benefit from approaches using simpler equipment, such as loop-mediated isothermal amplification assays. An alternative approach to genome detection is antigen detection mainly targeting CCHFV nucleoprotein (NP). Antigen detection assays can accurately diagnose CCHF infections but have a lower sensitivity than qRT–PCR assays[178]. Another alternative is virus isolation, but this process is time-consuming and requires appropriate biocontainment that can only be offered at certain reference laboratories.

Methods of choice for the detection of the host humoral immune response to CCHFV infections are largely based on enzyme-linked immunosorbent assays and indirect immunofluorescent assays. Serological assay kits for the detection of human IgM and IgG antibodies to CCHFV are commercially available. As antibody production can be delayed or even absent in severe CCHF cases, serological diagnosis of CCHFV may give false negatives in severe cases[91]. Thus, serological diagnosis is of greater use towards the end of the first week of illness and thereafter. Neutralization assays are of confirmatory diagnostic value. CCHFV-based neutralization assays require high biocontainment; however, assays based on pseudotype viruses or virus-like particles expressing the CCHFV glycoproteins[154] may allow for much wider use and easier handling at lower biocontainment.

CCHFV laboratory diagnostics are often performed in national or international reference laboratories, but the capacity for on-site testing helps to reduce delays for case management and public health intervention. The performance of the diagnostic laboratory should be regularly controlled and evaluated through participation in external quality assessment panels. Past experiences have identified performance weaknesses related to sensitivity and specificity of qRT–PCR assays[177], indicating a continuing need for improved performance and standardized protocols.

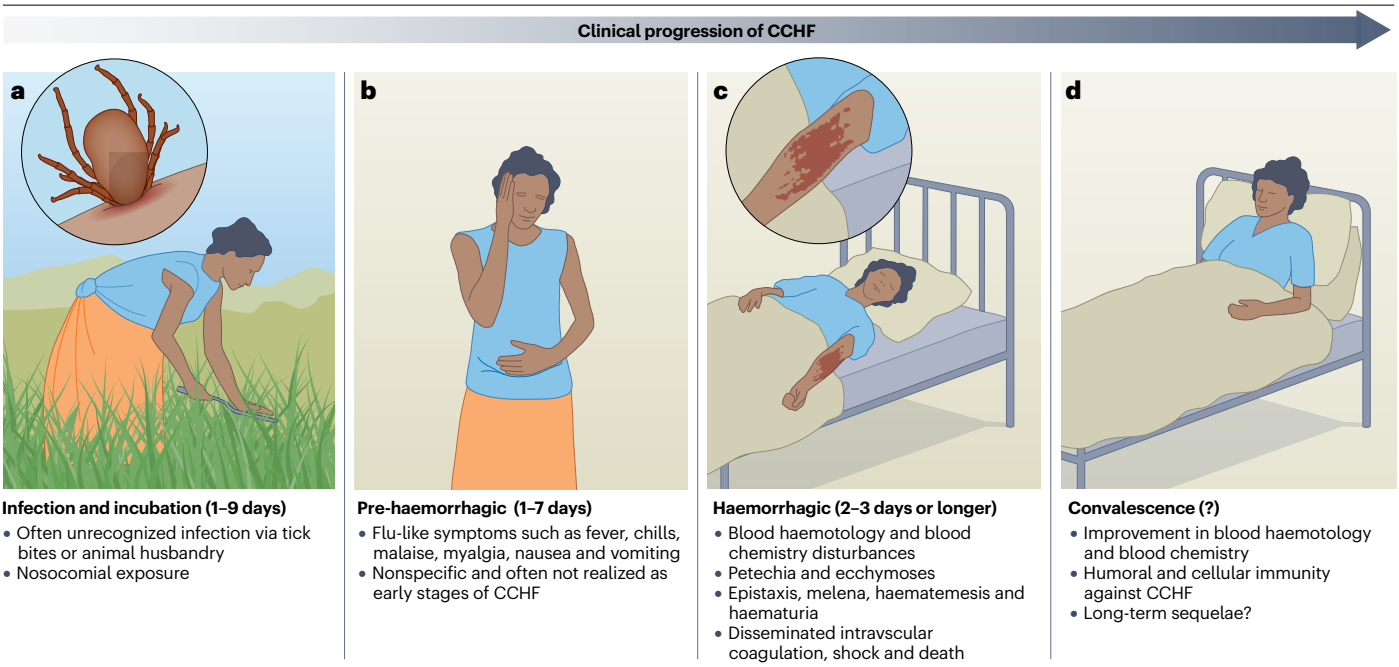


Fig. 2 | Clinical progression of Crimean–Congo haemorrhagic fever. The clinical progression of Crimean–Congo haemorrhagic fever (CCHF) presents with four distinct stages. Initial CCHF virus (CCHFV) infection is often unrecognized following exposure via tick bites or animal husbandry (part **a**), but transmission may also occur via nosocomial or intrafamily transmission during the care of sick patients. After infection, the incubation period (part **a**) may be as short as 1–3 days depending on the route of exposure. After the incubation period, infected humans may progress to a pre-haemorrhagic stage characterized by nonspecific symptoms such as fever, malaise, myalgia and nausea (part **b**). This pre-haemorrhagic phase of disease can then rapidly progress to haemorrhagic disease in the first week after infection (part **c**). This phase of disease is characterized by uncontrolled bleeding, liver damage, inflammatory immune responses and, in severe cases, disseminated intravascular coagulation, shock and death. In patients who survive, recovery begins 10–14 days after infection and is associated with return to normal of blood chemistry and haematology and development of anti-CCHFV immunity (part **d**). Long-term sequelae following CCHFV infection are poorly studied.

In a separate study evaluating infection with the same strains, more severe disease was reported, with animals developing clinical disease such as fever, viraemia, increased liver enzymes, thrombocytopenia and occasional rash and vaginal bleeding[135]. Again, no animals reached euthanasia criteria[135]. Interestingly, evidence of CCHFV persistence was found in the testes and latent tuberculosis granulomas of some CCHFV-infected macaques[135], suggesting that CCHFV may persist in immune-privileged sites.

It is currently unclear what accounts for the disease variability, but the outbred genetics of cynomolgus macaques, differences in virus strains used and institutional variation in euthanasia criteria may account for variable disease and ultimate outcome. Nevertheless, the cynomolgus macaque model accurately recapitulates many aspects of human disease and represents an essential model for preclinical evaluation of anti-CCHFV therapeutics and vaccines.

Tick models. To date, animal models have overwhelmingly used needle-delivered CCHFV and omitted the contribution of the tick and tick-derived factors to CCHFV pathogenesis[4]. Like many other arthropod-borne viruses, CCHFV must circulate and be maintained in both tick and vertebrate hosts. This life cycle places constraints on viral evolution (reviewed in ref. [136]) and may affect virulence[137]. Although models of tick feeding in high containment have been developed[42], much remains unknown about the role and contribution of the tick to CCHF and CCHFV pathogenesis.

Treatments for CCHF

Therapeutic options for CCHFV remain limited and most have focused on interfering with viral replication or modulating the host response to the infection (Table 1). Although many candidates have shown promising preclinical data, clinical efficacy data for most remain limited.

Antivirals

To date, the nucleoside analogue ribavirin is the only direct-acting antiviral that has been widely used clinically in patients with CCHF. However, the use of ribavirin is controversial, with continued debate about whether treatment improves outcome[63,77,138–144]. Systematic meta-analyses indicate that the data supporting the efficacy of ribavirin against CCHFV are poor owing to confounding factors in reported data sets[144,145], and any benefit probably requires early treatment[146]. Animal studies have also shown conflicting efficacy of ribavirin against CCHFV infection. Although ribavirin was effective in lethally infected *Stat1*$^{-/-}$ mice[110] and *Stat2*$^{-/-}$ hamsters[112], two studies in lethally infected *Ifnar*$^{-/-}$ mice showed no protection against death even with prompt treatment[114,116]. Cumulatively, the data from humans infected with CCHFV and from animal models suggest that ribavirin has poor efficacy against CCHFV and that any benefit probably requires prompt treatment following known exposure. This is difficult to achieve outside recognized laboratory or health-care exposure.

In contrast to ribavirin, favipiravir or a derivative (H44) showed significant protective effects in lethally infected mice[114,116,147], preventing

death and significantly reducing viral loads in key target tissues of CCHFV. Favipiravir or H44 treatment could even be initiated days after infection[114,147], including when mice were exhibiting advanced signs of disease, and still provide significant protective effects[116]. These data suggest that favipiravir and related compounds may be effective in patients presenting to health-care systems with advanced CCHF. Interestingly, lethal recrudescent CCHFV infection was observed weeks after cessation of favipiravir treatment in infected mice[116], suggesting that early favipiravir treatment may not completely control the virus. Favipiravir was also effective in CCHFV-infected cynomolgus macaques, reducing viraemia and viral burden in several tissues[133]. Although favipiravir has shown promise in preclinical animal models, efficacy data in humans infected with CCHFV is limited[148] and clinical trials are needed to determine whether favipiravir can improve CCHF patient outcomes. 2′-Deoxy-2′-fluorocytidine has also shown promising results in vitro[115], suggesting that this may be another effective antiviral against CCHFV. Molnupiravir, recently used to treat SARS-CoV-2 infection in humans[149], exhibits efficacy against CCHFV in vitro with similar inhibitory concentrations as favipiravir[147]. Nevertheless, molnupiravir failed to protect against CCHFV infection in lethally infected mice even when treatment was started before infection[147].

Although ribavirin, favipiravir and 2′-deoxy-2′-fluorocytidine are all thought to exert antiviral activity through catastrophic mutagenesis or inhibition of the viral replicase[150], additional small molecules acting through distinct mechanisms have been reported effective against CCHFV in vitro[151]. TH3289, a compound with broad antiviral activity in vitro, has been shown to suppress CCHFV replication, probably by modulating interactions between viral proteins and cellular chaperone proteins[151]. Blockade of the catalytic activity of the CCHFV OTU domain with a synthetic ubiquitin variant was able to block CCHFV replication in vitro through interference with viral RNA synthesis[50]. However, further validation of these potential antivirals against CCHFV in vivo is needed.

Antibody-based therapies

In addition to small molecules, antibody-based therapies have also been evaluated for treatment of CCHFV (Table 1). Although large-scale trials have not been performed, limited evidence suggests that administration of plasma or antibodies from survivors of CCHF can confer a benefit in seriously ill patients (reviewed in refs. [152,153]). However, the scalability of convalescent plasma treatments is limited, and continued research has shown efficacy of mouse and human monoclonal antibodies against CCHFV in lethally infected mice. Evaluation of a panel of mouse monoclonal antibodies to the viral GPC showed that several neutralizing and non-neutralizing antibodies could protect lethally infected neonatal mice[33]. However, some antibodies that showed efficacy in the neonatal model failed to protect lethally infected adult type I interferon-deficient mice[40]. Potently neutralizing antibodies derived from human CCHF survivors failed to protect lethally infected mice in a post-exposure setting, although a hybrid bicistronic antibody was able to protect 80% of mice when administered 24 h post-infection[117]. As protection was incomplete even when mice were treated with a large amount (1 mg) of this antibody just 24 h post-infection, further studies are needed to define the therapeutic window of neutralizing antibodies for treatment of CCHF. Although the CCHFV GPC is the most diverse segment, a subset of antibodies was found to exhibit broad neutralization activity, demonstrating that there are conserved neutralizing epitopes across divergent strains of CCHFV[154]. Interestingly, antibody-mediated protection against CCHFV does not require neutralizing activity, and several antibodies targeting non-neutralizing epitopes in the CCHFV GP38 protein were found to be protective in animal models[33,40]. The protective efficacy of the GP38-targeting monoclonal antibody was found to require complement activity, suggesting that antibody effector functions such as complement-mediated lysis or viral opsonization and phagocytosis may be critical for antibody-mediated protection against CCHFV[40].

Anti-inflammatory drugs

Severe CCHF, like many haemorrhagic fevers, involves a dysregulated inflammatory response and cytokine storms leading to substantial immunopathology. Thus, limited attempts have been made to use anti-inflammatory drugs in patients with CCHF to suppress the hyperinflammatory host response. In a comparative study of patients with confirmed CCHF, high-dose methylprednisolone with ribavirin improved outcomes compared with ribavirin alone[155]. Corticosteroids

Table 1 | Treatments for Crimean–Congo haemorrhagic fever

Compound	Class	Target	Preclinical efficacy	Clinical efficacy	Comments	Refs.
Ribavirin	Nucleoside analogue	RdRP	Controversial efficacy in rodent models	Controversial efficacy in patients	Poor efficacy; early treatment start needed; should be discontinued or used in combination therapy	110,114,116,145
Favipiravir	Nucleoside analogue	RdRP	Efficacy in rodent and NHP models	Limited data or benefit	Late treatment start effective in rodent models; clinical trials are needed	114,116,147,148
2′-Deoxy-2′-fluorocytidine	Nucleoside analogue	RdRP	Not done	No clinical data	More preclinical studies are needed	115
Molnupiravir	Nucleoside analogue	RdRP	No efficacy in rodent models	No clinical data	Unlikely to proceed	147
Plasma or antibodies from survivors	Neutralizing or non-neutralizing	Viral proteins	Not done	Limited data or benefit	More preclinical and/or clinical studies are needed	153
Monoclonal antibodies	Neutralizing or non-neutralizing	Viral proteins	Limited data in rodent models	No clinical data	More preclinical and/or clinical studies are needed	33,40,117
Corticosteroids	Anti-inflammatory	Host response	Not done	Limited data or benefit	More preclinical and/or clinical studies are needed	84,155

NHP, non-human primate; RdRP, RNA-dependent RNA polymerase.

also seemed to have a benefit in severely ill patients[84]. However, the cohort size of these studies is limited. A recent study in lethally infected type I interferon-blockaded mice showed that infection of mice lacking the TNF receptor or treatment with an antibody to block TNF signalling could protect against lethal disease[119]. The availability of clinically approved TNF therapeutics[156] and therapeutics against other host cytokines[157] may warrant evaluation of this approach to treat CCHF.

Prevention and vaccines

Although antivirals and antibody-based therapies for CCHFV have shown promise in preclinical models, the utility of these treatments is limited to well-developed health-care systems with the ability to rapidly recognize and diagnose CCHFV infections, access to the drugs and ability to promptly begin treatment. Therapies for patients in countries with limited health-care resources or presenting to health-care systems when exhibiting advanced disease are likely to remain limited. Consequently, public health education to prevent exposure to CCHFV and vaccines are critically needed to address the public health threat of CCHFV infections in areas with limited access to health care.

Prevention

Preventing CCHFV infection involves addressing the many risk factors for CCHFV exposure (Fig. 3). For farmers, wearing appropriate clothing such as long sleeves and pants (Fig. 3a), reducing activities in tick-infested areas and use of integrated pest management strategies (Fig. 3b) to reduce tick populations in the farm environment can minimize risk of CCHFV infection via tick bites (reviewed in ref. [158]). Furthermore, using protective equipment when slaughtering tick-invested livestock either in backyard slaughter processes or in abattoirs may reduce exposure via contaminated animal products (Fig. 3a). In the health-care setting, personal protective equipment is essential to prevent transmission during care of patients infected with CCHFV[67,159] (Fig. 3a). Educational campaigns to inform people in endemic areas of the risk factors for CCHF (such as tick bites and workplace dangers) may prompt at-risk populations to reduce their risk of exposure and to recognize and report early symptoms of CCHF (Fig. 3d). Quarantine of livestock potentially carrying CCHFV or CCHFV-infected ticks before transport or slaughter may also prevent exposure and limit the introduction of CCHFV into new areas (Fig. 3e).

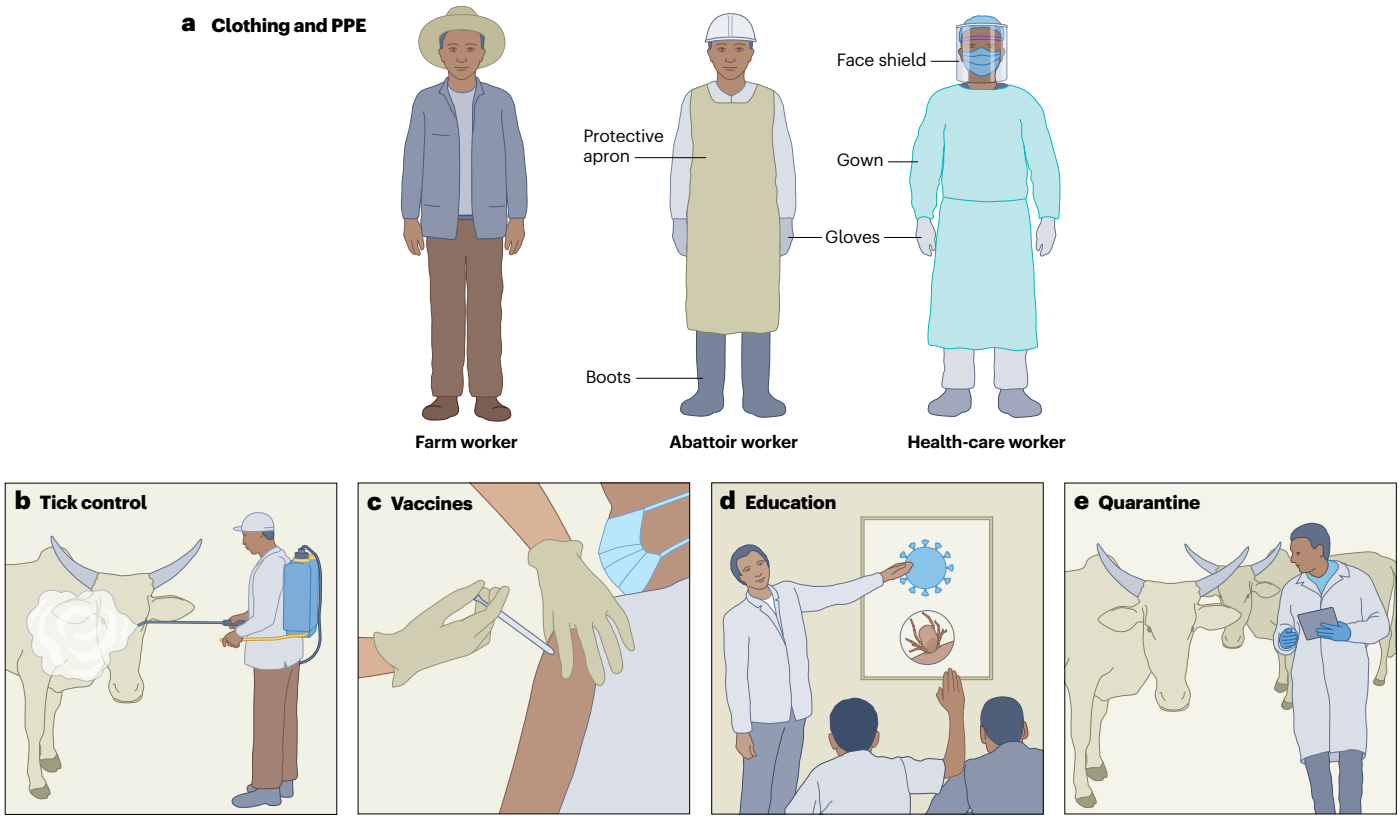


Fig. 3 | Prevention of Crimean–Congo haemorrhagic fever. Prevention of Crimean–Congo haemorrhagic fever (CCHF) requires a multifactorial approach addressing public and veterinary health. **a**, Farm workers should wear long sleeves and pants to limit tick bites. Transmission of CCHF in the health-care and abattoir settings can be limited by wearing of proper personal protective equipment (PPE). Standard barrier PPE such as a laboratory gown, gloves, a face shield and a mask can limit exposure of health-care personnel to contaminated bodily fluids. Abattoir workers can be similarly protected by wearing PPE such as gowns and gloves and receiving proper training on how to butcher animals. **b**, Control of the tick vector is also important and may include deployment of acaracides to control tick populations on the farm and on livestock. **c**, Vaccines are also critically needed to prevent disease on CCHF virus (CCHFV) infection and could also be deployed in livestock populations to interrupt the CCHFV life cycle. **d**, Public health-directed educational efforts can help to inform at-risk populations to reduce activities that expose them to CCHFV-infected ticks or livestock, to recognize the risk of tick bites in transmission of CCHFV and to promptly recognize the early symptoms of CCHFV. **e**, Inspection and quarantine of animals moving from CCHFV-endemic areas can reduce exposure of humans to CCHFV-infected ticks and prevent introduction of CCHFV to new areas.

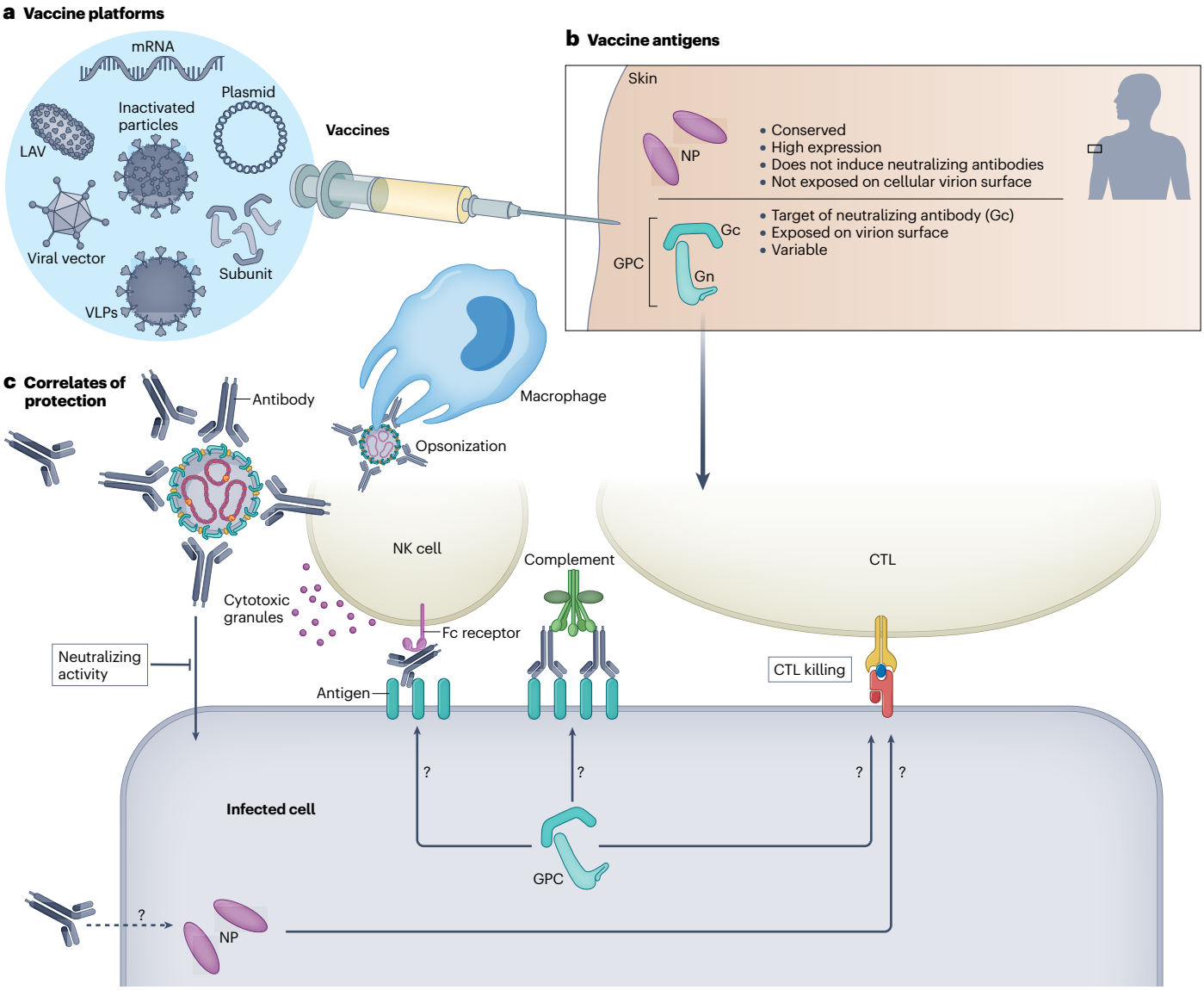


Fig. 4 | Vaccines for Crimean–Congo haemorrhagic fever virus. Multiple vaccine platforms for Crimean–Congo haemorrhagic fever virus (CCHFV) have reached various stages of preclinical testing and include nucleic acid-based vaccines such as plasmid DNA and mRNA, inactivated virus preparations, live-attenuated vaccines (LAVs) such as recombinant vesicular stomatitis virus, virally vectored vaccines such as adenovirus-vectored vaccines, virus-like particles (VLPs) and subunit vaccines with purified viral proteins (part **a**). The viral antigen expressed by these vaccines is likely to be important (part **b**), with protective efficacy demonstrated by vaccines expressing nucleoprotein (NP) and/or the glycoprotein precursor (GPC). Importantly, the correlates of protection for vaccines against CCHFV are unknown (part **c**) and neutralizing antibody is dispensable. Thus, vaccine-mediated protection may require effector functions such as viral opsonization, complement activation or antibody-dependent cellular cytotoxicity by natural killer (NK) cells. In studies demonstrating the protective efficacy of antibodies to the CCHFV NP alone, it remains unclear how antibodies to NP can protect. The role of cellular immunity such as cytotoxic T cell-mediated cytotoxicity is similarly unknown. CTL, cytotoxic T lymphocyte.

Vaccines

To date, multiple vaccine platforms have been evaluated in animal models for CCHFV such as inactivated virus preparations[160], subunit vaccines[161], VLPs[162,163], recombinant live-attenuated viruses[164,165], replication-deficient viral-vectored vaccines[166] and nucleic acid-based vaccines[167–171] (Fig. 4a), many with promising efficacy. These vaccine approaches to CCHFV have been extensively reviewed elsewhere[118,172]. However, the vaccine landscape for CCHFV is complex: vaccine-induced neutralizing antibodies seem neither necessary nor sufficient for protection, several viral antigens can confer protection, and yet the same viral antigens expressed from different vaccine platforms can confer distinctly different levels of efficacy.

Preclinical studies have shown protection with vaccines expressing the CCHFV NP, GPC or just the glycoproteins Gn and Gc (Fig. 4b), demonstrating that there are multiple protective epitopes within CCHFV. Within these antigens, the specific protective epitopes are unclear.

Mice vaccinated with a DNA plasmid expressing just the CCHFV Gn and Gc but lacking the MLD and GP38 developed anti-CCHFV antibodies as measured by enzyme-linked immunosorbent assay (ELISA) but were not protected against lethal CCHFV infection[173]. Yet, immunity to GP38 alone was poorly protective[173]. The epitopes targeted by NP-directed immunity are unknown.

To avoid the need for developing strain-specific or region-specific vaccines, a vaccine for CCHFV must confer protection against genetically diverse strains of CCHFV. The genetic diversity of CCHFV is a concern as a DNA vaccine expressing the CCHFV GPC showed incomplete protection when mice were challenged with a heterologous strain of CCHFV[173], indicating that viral escape due to the genetic diversity of CCHFV is possible. By contrast, vaccines based on replicating RNA (repRNA), vesicular stomatitis virus and VLP have shown heterologous protection[162,164,171]. However, many vaccines evaluated in preclinical models have only evaluated homologous challenge, and it is unclear how broad or narrow the protection is that is conferred by these vaccines. Alternatively, most of the genetic diversity of CCHFV is located within the M segment encoding for the GPC. Vaccines including the more conserved viral NP have shown significant efficacy[162,163,166,168,169], and vaccines expressing just NP can confer robust protection[166,169,171,174], suggesting that inclusion of NP may avoid viral escape of vaccine-conferred immunity.

The correlates of vaccine-mediated protection against CCHFV are poorly understood and seem to be less straightforward than the levels of vaccine-induced neutralizing antibodies (Fig. 4c). Notably, vaccines based on repRNA, mRNA and DNA showed significant protection in mice or NHPs in the absence of detectable neutralizing antibodies following vaccination[163,168,169,171]. By contrast, subunit and VLP-based vaccines induced high levels of neutralizing antibodies but failed to protect lethally infected mice[161,163], and protection did not correlate with neutralizing titres in comparisons between VLP-vaccinated and DNA-vaccinated mice[163]. Although robust ELISA titres were measured in mice vaccinated with a DNA vaccine expressing Gn and Gc but lacking MLD and GP38, mice were not protected against lethal challenge[173]. Yet, in mice vaccinated with a repRNA vaccine, studies in B cell-deficient mice have demonstrated that protection required humoral immunity[171]. Together, these data demonstrate that although humoral immunity is important, neutralizing antibodies are neither necessary nor sufficient for vaccine-mediated protection against CCHFV. These data also demonstrate that ELISA and neutralizing titres may not be sufficient to predict vaccine efficacy and that a better understanding of the correlates of protection for CCHFV is needed, an important consideration as vaccine platforms move into clinical trials.

These vaccine data add to observations that human survivors often do not develop neutralizing antibodies until well after resolution of clinical disease[91] and that non-neutralizing antibodies can be effective therapeutics in preclinical models[33]. Thus, the functional requirement of vaccine-induced antibodies to CCHFV for protection seems complex and may require effector functions such as complement activation or antibody-dependent cellular cytotoxicity (Fig. 4c). In studies with repRNA-vaccinated mice, robust protection against CCHFV was correlated with antibody responses against the CCHFV NP, yet this antigen was not accessible on intact infected cells in vitro[171], arguing against mechanisms such as antibody-dependent cellular cytotoxicity and complement activation for vaccine-mediated control of the infection. NHPs vaccinated with a plasmid expressing just the NP had significant protection following CCHFV challenge[174], demonstrating that the protective efficacy of the NP antigen is not an artefact of the mouse models. How antibodies to an internal CCHFV protein can confer protection requires further study (Fig. 4c). Similarly, the role of vaccine-induced cellular immunity in protection against CCHF is unclear. Protection required transfer of both antibody and T cells from modified vaccinia Ankara-vaccinated mice[175], suggesting that both humoral and cellular immunity contribute to vaccine-mediated protection. By contrast, in mice vaccinated with repRNA, depletion of T cells at the time of viral challenge did not alter survival, suggesting that cellular immunity is dispensable for protection[171]. Although multiple vaccines have been shown to elicit cellular immunity in animal models[118], the requirement of cellular immunity for protection remains largely untested and may be vaccine-specific. It is also unknown what effector functions are required for protection.

The vaccine platform may also be an important consideration for CCHFV. A modified vaccinia Ankara vaccine expressing the CCHFV NP failed to protect mice despite eliciting both humoral and cellular immunity[176]. By contrast, an adenovirus vector expressing the NP was partially protective[166], whereas an mRNA or repRNA expressing the NP conferred 100% protection[169,171]. These data demonstrate that the same vaccine-expressed antigen can have differing protective effects depending on the vaccine platform and that varied efficacy may be due to distinct vaccine-induced immune responses. Mechanistic studies investigating how CCHFV vaccine candidates protect in animal models are urgently needed to define the immune responses and their effector functions required for protection. These data are needed to refine candidate vaccines to drive protective immunogenicity and define the immune responses that must be measured in human clinical trials to monitor and predict vaccine efficacy.

Conclusions

Despite a wide geographical range and large populations at risk for infection with CCHFV, much remains to be determined regarding the host and viral determinants of CCHFV pathogenesis. Novel functions of viral proteins probably remain to be discovered, and the development of molecular virology tools and improved small-animal models will enable further mechanistic insight into how CCHFV causes disease. For at-risk populations, preventive measures such as education, reduced tick contact, treatment of livestock to control tick infestations, livestock quarantine and protection for high-risk exposure activities need to be implemented in endemic areas. Importantly, rapid and reliable diagnostics along with efficacious vaccines and antivirals are needed to limit the burden of CCHF on patients and public health-care systems. Given that vaccines may protect against CCHFV through mechanisms other than classical antibody-mediated neutralization, investigating how vaccines protect against CCHFV will provide insight into how the host can control the infection and inform treatment strategies that promote effective immune responses while limiting immunopathology. Cumulatively, continued contributions from the fields of molecular virology, immunology, vaccinology, entomology, veterinary health and public health will be needed to address the substantial risk of CCHFV infection and disease in endemic areas.

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