

Hamster models of COVID-19 pneumonia reviewed: How human can they be?

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Abstract

The dramatic global consequences of the coronavirus disease 2019 (COVID-19) pandemic soon fueled quests for a suitable model that would facilitate the development and testing of therapies and vaccines. In contrast to other rodents, hamsters are naturally susceptible to infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the Syrian hamster (*Mesocricetus auratus*) rapidly developed into a popular model. It recapitulates many characteristic features as seen in patients with a moderate, self-limiting course of the disease such as specific patterns of respiratory tract inflammation, vascular endothelialitis, and age dependence. Among 4 other hamster species examined, the Roborovski dwarf hamster (*Phodopus roborovskii*) more closely mimics the disease in highly susceptible patients with frequent lethal outcome, including devastating diffuse alveolar damage and coagulopathy. Thus, different hamster species are available to mimic different courses of the wide spectrum of COVID-19 manifestations in humans. On the other hand, fewer diagnostic tools and information on immune functions and molecular pathways are available than in mice, which limits mechanistic studies and inference to humans in several aspects. Still, under pandemic conditions with high pressure on progress in both basic and clinically oriented research, the Syrian hamster has turned into the leading non-transgenic model at an unprecedented pace, currently used in innumerable studies that all aim to combat the impact of the virus with its new variants of concern. As in other models, its strength rests upon a solid understanding of its similarities to and differences from the human disease, which we review here.

Keywords

animal model, COVID-19, hamster, histopathology, review, pathology, preclinical research, severe acute respiratory syndrome coronavirus 2, vaccines

Among the most urgent challenges early in the coronavirus disease 2019 (COVID-19) pandemic was the immediate search for an appropriate and robust model that would allow understanding of mechanisms of the disease, to develop targeted therapies, and to test antiviral drugs and vaccines against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Several 2- or 3-dimensional tissue culture models and organoids derived from human lung, bronchi, alveoli, blood vessels, and other tissues were established which are helpful for specific mechanistic studies but have strong limitations for more complex study goals.⁶⁸ Particularly for purposes where fully differentiated cells and tissues, complete immune functions, or the systemic circulation are required, animal models still remain indispensable. But which animal species would be capable of optimally modelling human COVID-19 with its wide spectrum of disease manifestations and challenges?

As soon as angiotensin converting enzyme-2 (ACE2) was discovered as the principle human cellular receptor for the viral spike (S)-protein, systematic structural protein analyses of mammalian ACE2 homologs predicted that macaques, mink, ferrets, common marmosets, felines, rabbits, hamsters, and few

other mammals but not mice would be naturally susceptible to the infection,^{17,23,99,101} which was soon confirmed by experimental infections.^{13,23,24,42,44,58,61,62,70,77} Among these species, and mainly for practical reasons, the Syrian or golden hamster (*Mesocricetus auratus*), which had already served as a model for SARS-CoV infection in 2005, soon became a popular, widely used, and well-characterized small animal model for COVID-19-associated pneumonia.^{58,67,68,70}

To facilitate experimental infections in mice as an affordable and highly standardized *in vivo* model for large-scale laboratory studies, mouse-adapted strains of SARS-CoV-2 and

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hybrid SARS-CoV/SARS-CoV-2 strains were developed but did not reach wide practical relevance.^{21,101} At the same time, various genetically modified mouse models were established that expressed the human ACE2 receptor (hACE2) to facilitate entry of SARS-CoV-2 into murine cells, such as via transgene or knock-in technologies using universal or cell type-specific promoters. Also, infections with recombinant adenoviral or adenovirus-associated viral vectors were used.^{21,24,41,58,75,93} However, the outcome of infection and lesions observed in murine models largely depended on the transgene technology used, with model-specific variations in hACE2 expression levels and expressing cell types. For example, the neuroinvasion and encephalitis observed in the widely used K18-hACE2 mouse, which expresses hACE2 under expressional control of the cytokeratin 18 promoter, appears to be rather specific for this model with little comparative value for human patients.^{21,50} Despite several advantages of mice as standard laboratory animals, including the accessibility of vast molecular information and research tools, available mouse models for COVID-19 research are therefore considered more artificial than naturally susceptible species such as hamsters.^{13,21,36,52,58,68}

Here, the general question arises as to what can be expected from a small rodent, particularly a hamster, in the modelling of human COVID-19. The universal translatability of observations obtained from mice and other rodents to humans, in particular in pneumonia research, is limited for various reasons, including species-specific differences in their anatomies and respiratory physiologies, cellular target structures, immune functions, and microbiome status.^{56,57} Particular challenges are posed for the modelling of a highly multifaceted disease such as COVID-19 with its extraordinarily wide spectrum of consequences, including diverse clinical courses and outcomes of pneumonia and vascular disorders in different groups of society.^{11,12} As for any animal model of human disease, it is of utmost importance to first define a specific aim and to ensure that the chosen model is fit-for-purpose.²⁶ So, how human can a hamster be for understanding disease mechanisms and in the development as well as testing of antiviral drugs, mechanistic therapies, and new vaccines geared toward fighting the pandemic? This core question will be addressed at the end of this review, after summarizing what is currently known of this model and its similarities to and differences from human COVID-19.

Target Cells, Route, Distribution, and Clearing of the Virus

Several studies using experimental transnasal inoculation with SARS-CoV-2 of 4- to 8-week-old Syrian hamsters have yielded similar results of a transient, self-limiting, epitheliotropic infection of the lungs and intestine with almost complete elimination of the virus before 14 days post infection (dpi). Most groups have used 10^3 to 10^5 plaque-forming units (pfu); similar to tissue culture infectious dose 50, TCID₅₀) of the virus with only 5 pfu identified as a minimal dose to establish a productive infection.⁷⁰ One group also included the intraocular route of

infection.⁴⁴ In addition to virus isolation and reverse transcriptase polymerase chain reaction (RT-PCR)-based detection of the virus, immunohistochemistry (IHC), immunofluorescence, or in situ hybridization (ISH) have been used, mostly to localize the viral nucleocapsid (N) protein or RNA encoding the N or S protein, respectively, in histological sections.^{18,44,61,70,77} The results were largely identical regardless of whether the viral N protein or viral RNA encoding either the N or S protein was detected. As viral spread and tissue lesions proceed somewhat dysphasically, with the most obvious histological lesions lagging behind the actual infection, they will be discussed separately here.

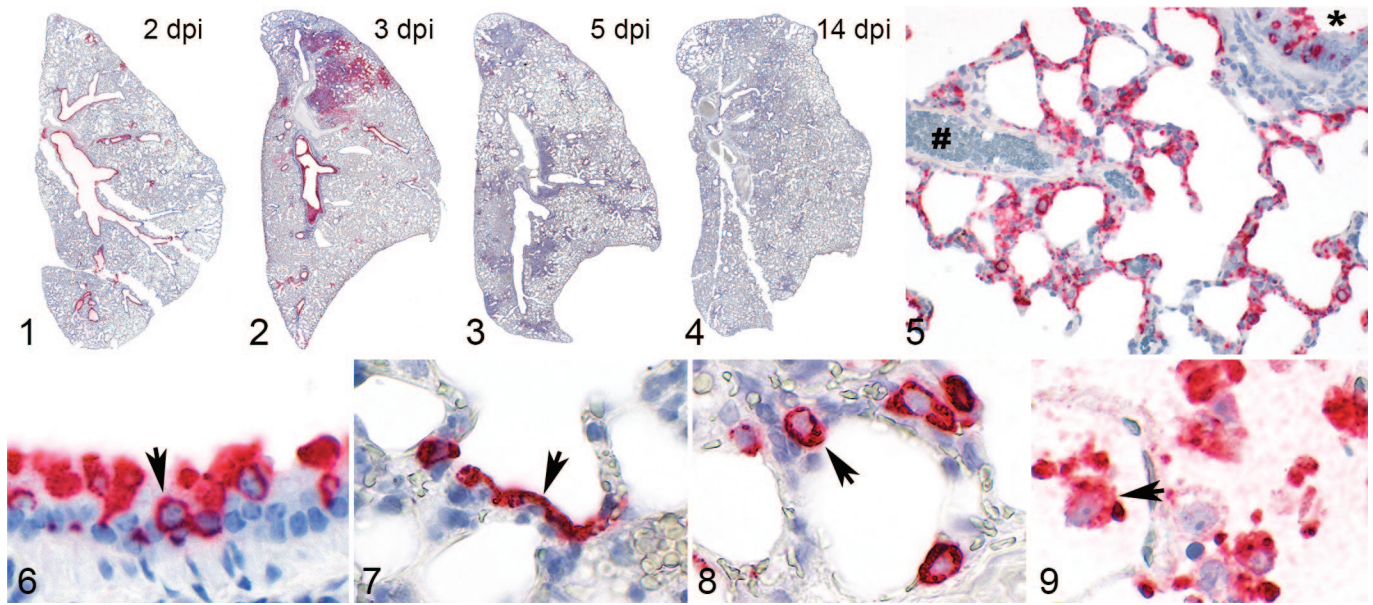
For a better understanding of both the distribution of the virus and histologic lesions, the anatomy of the hamster respiratory tract and its minor differences to that of mice and rats are compiled in detail elsewhere.⁶⁶ However, these are obviously largely negligible in this context as none of the reports reviewed here actually mentioned hamster-specific anatomies.

All studies consistently point toward an airway-associated spread of the virus from the upper nasopharyngeal and respiratory epithelium into the lungs, and here in a patchy pattern spreading centrifugally from the hilus along the airways into the alveoli (Figs. 1–4). In general, the susceptibility of target cells as identified by ISH correlates with cellular expression patterns of its ACE2 receptor, with strongest expression on ciliated epithelial cells and alveolar type II epithelial cells (AEC-II) in hamsters (Figs. 5–8).^{59,61,83} The role of alternative mediators of cellular entry by SARS-CoV-2 such as CD147, neuropilin-1 (NRP1), and myeloid C-lectin-like receptor (CLR) has not yet been investigated in hamsters.⁵⁵

At 2 dpi, large numbers of high columnar ciliated respiratory epithelial cells are infected throughout the nasal cavity, mostly along the nasal turbinates, and in few submucosal glands.^{23,77} Viral antigen can also be found in the vomeronasal organ, the olfactory epithelium (OE), and in the lateral nasal (Steno's) gland.¹⁸ At the same time, single or clusters of tracheal and bronchial ciliated and secretory epithelial cells, some of which detached, contain high amounts of the virus.^{44,61,70,77} In contrast to humans, however, viral antigen-expressing tracheal and bronchial epithelial (BE) cells in hamsters have not yet been subclassified in detail as club cells, brush cells, goblet cells, or progenitor cells.⁷² Also at 2 and 3 dpi, distal bronchiolar epithelial cells as well as alveolar type I epithelial cells (AEC-I) and AEC-II are infected.^{23,44,61,70,77}

The shedding of infectious viral particles via nasal secretions and oropharyngeal discharge peaks between 3 and 5 dpi and is terminated by the appearance of neutralizing antibodies approximately 1 week after infection.^{70,101} Consequently, transmission between co-housed animals can occur effectively via direct contact or aerosol during this time.⁷⁷

Important for the immunopathogenesis of the disease in hamsters, large amounts of virus can also be detected in high numbers of intrabronchial and alveolar macrophages from 2 to 7 dpi (Fig. 9), likely a consequence of their phagocytic activity rather than direct infection.⁵⁹ In fact, copy numbers of viral



Figures 1–9. SARS-CoV-2 infection, lung, Syrian hamster. In situ hybridization (ISH) for viral RNA (red chromogen, blue hemalaun counterstain). **Figure 1.** At 2 days post infection (dpi), ISH localizes the virus only in bronchial epithelium throughout the entire left lung lobe. **Figure 2.** At 3 dpi, viral infection spreads to the respiratory parenchyma in a patchy pattern and the bronchial epithelium is still strongly infected. **Figure 3.** At 5 dpi, the infection turns into a purely parenchymal pattern with less signal. **Figure 4.** Viral RNA was no longer detected at 14 dpi. **Figure 5.** At 3 dpi, there is almost complete infection of the alveolar lining cells in affected areas as well as patchy infection of bronchial epithelial cells (asterisk). #: a blood vessel with virus-negative endothelial cells. **Figures 6–9.** Viral RNA is localized in the cytosol of bronchial epithelial cells (Fig. 6, arrow), alveolar epithelial cells (AEC) type-I (Fig. 7, arrow), AEC-II (Fig. 8, arrow), and alveolar macrophages (Fig. 9, arrow). Methods were described earlier.^{61,87}

RNA per cell in alveolar monocyte-derived macrophages are higher than in any other cell type.⁵⁹ At all times throughout the infection, both viral proteins and RNA can be consistently localized to the cytosol of epithelial cells and macrophages, the latter often with granular, punctate staining patterns indicative of phagocytized cellular debris and uptake of cell-free virus.^{59,70}

At 4 dpi, mostly detached epithelial cells are virus-positive in the trachea, suggesting clearance of the virus with regeneration of remaining tracheal epithelial cells.²³ In more distal airway epithelia, however, and all over the alveolar walls, the virus is found more abundantly than at 2 dpi. Viral loads in AEC-I, AEC-II, alveolar macrophages, and cellular debris in alveolar spaces peak around 3 dpi (Figs. 5–9). Comparisons of inoculations with 10^3 or 10^5 pfu resulted in clear dose-dependent differences in viral loads at 3 dpi but not at 6 dpi or later.^{44,70} Clearance of the virus from viable airway epithelial cells and from the majority of AEC occurs by 6 or 7 dpi with virus detectable only in macrophages and cellular debris in alveolar spaces and airways.^{23,44,70,77} At 10 or 14 dpi, virus was cleared from the entire respiratory tract in virtually all studies.

Loss of gustatory and olfactory functions which are hallmarks of the human disease are well reproduced in SARS-CoV-2-inoculated Syrian hamsters. Both ageusia and anosmia were recorded between 2 and 5 dpi.²⁵ However, different reports had contradictory findings on direct infection of

olfactory neurons (ON) within the OE, which have been postulated to allow virus entry into the brain. Two studies co-localized the viral N protein in the OE with a strictly neuron-specific marker, and virus particles were observed to bud from ON by scanning electron microscopy.^{25,77} One of these studies also localized the viral N protein along axons of ON reaching the olfactory bulb, with infection of cells with neuronal and glial morphology in the glomerular layer of the olfactory bulb but not beyond.²⁵ In contrast, another study failed to detect the virus in ON labeled by a different neuron-specific marker or in the olfactory bulb, olfactory cortex, or other regions of the hamster brain. Instead, at 2 dpi high amounts of the virus were localized to sustentacular cells which serve glial cell-like functions in the OE, with rapid clearance of the virus after 4 dpi.¹⁸ Infection of the OE sustentacular cells was accompanied by marked lymphocytic infiltration and damage of the OE at 2 and 4 dpi with almost complete recovery by 14 dpi.

Although pulmonary vascular endothelial cells play a hallmark role in the pathogenesis of the disease both in humans and hamsters,⁵⁹ they were not found to be infected in hamsters as detected by IHC, ISH (Fig. 5), or single cell RNA-sequencing (scRNA-Seq).^{5,59,61} Viremia seems to be low, if any, and occurs in only few animals.^{23,59,61}

By and large, the spectrum of cell types infected in Syrian hamster airways seems to widely overlap with the spectrum of infected epithelial cells and macrophages as described in

human COVID-19,^{11,72,74} with the limitation that data from the acute stage in humans are obviously scarce. In terms of similarities and differences in the expression patterns of the cellular receptor and required cofactors, available data are also incomplete for cross-species comparisons, partly due to their low expression levels below the sensitivity threshold of commonly used IHC and ISH protocols. This is true for ACE2 and the transmembrane serine protease 2 (TMPRSS2), which are required for viral entry and spread, as well as for the endoprotease furin.^{43,59,70,74,83}

Outside the respiratory tract, SARS-CoV-2 has been localized to single or clusters of small and large intestinal epithelial cells in Syrian hamsters with little or no evidence of tissue damage or inflammation,^{23,80} which is similar to observations in COVID-19 patients.⁵⁴ The enteric path of the virus including its silent replication in the intestinal epithelium may follow the swallowing of cellular airway debris, however, with unknown consequences. Feces may contain viral RNA but the relevance of fecal transmission between animals remains questionable.⁷⁷

Aside from sporadic detection in lymphoid organs, no extra-respiratory spread of SARS-CoV-2 has been observed in most Syrian hamster studies that used the standard infectious dose of 10⁵ pfu.⁷⁰ However, a more systematic multi-organ spread of the virus was observed in Syrian hamsters after using a rather high infectious dose of 10⁶ pfu, again via the transnasal route. Here, in addition to similar distributions in the respiratory tract as described above, viral protein and/or RNA were also detected at 7 dpi in the medulla and cortex of the adrenal glands, epithelial cells of the renal tubules, vesicular and prostate glands, corpora lutea of the ovary, myocardium, spleen, lymph nodes, liver, and gall bladder, albeit only in single animals for some organs.⁸⁰ Importantly, SARS-CoV-2 has consistently not been found in the brains of Syrian hamsters at relevant levels, regardless of the dose used for infection.^{23,44,70,80}

Clinical Signs

Virtually all reports indicate only moderate transient clinical signs in Syrian hamsters during the first days up to 2 weeks after infection, including lethargy, hunch back posture, ruffled fur, tachypnea, changes in respiratory patterns with rapid breathing, and transient body weight loss of up to 15% peaking around 6 to 7 dpi.^{23,44,61,70} Syrian hamsters do not usually succumb to disease caused by SARS-CoV-2 infection.

Micro-CT Analysis and Macroscopic Pathology

Micro-computed tomography (CT) of living hamsters was performed to compare intravital lesions with those reported for COVID-19 patients, where this diagnostic approach is commonly used. Micro-CT lung abnormalities in Syrian hamsters started at 2 dpi with ill-defined, patchy ground glass opacity with a central, peribronchial distribution and progressed to largely consolidated lungs peaking at 7 to 8 dpi.^{13,44} Different doses of the virus used for infection resulted in a clearly dose-

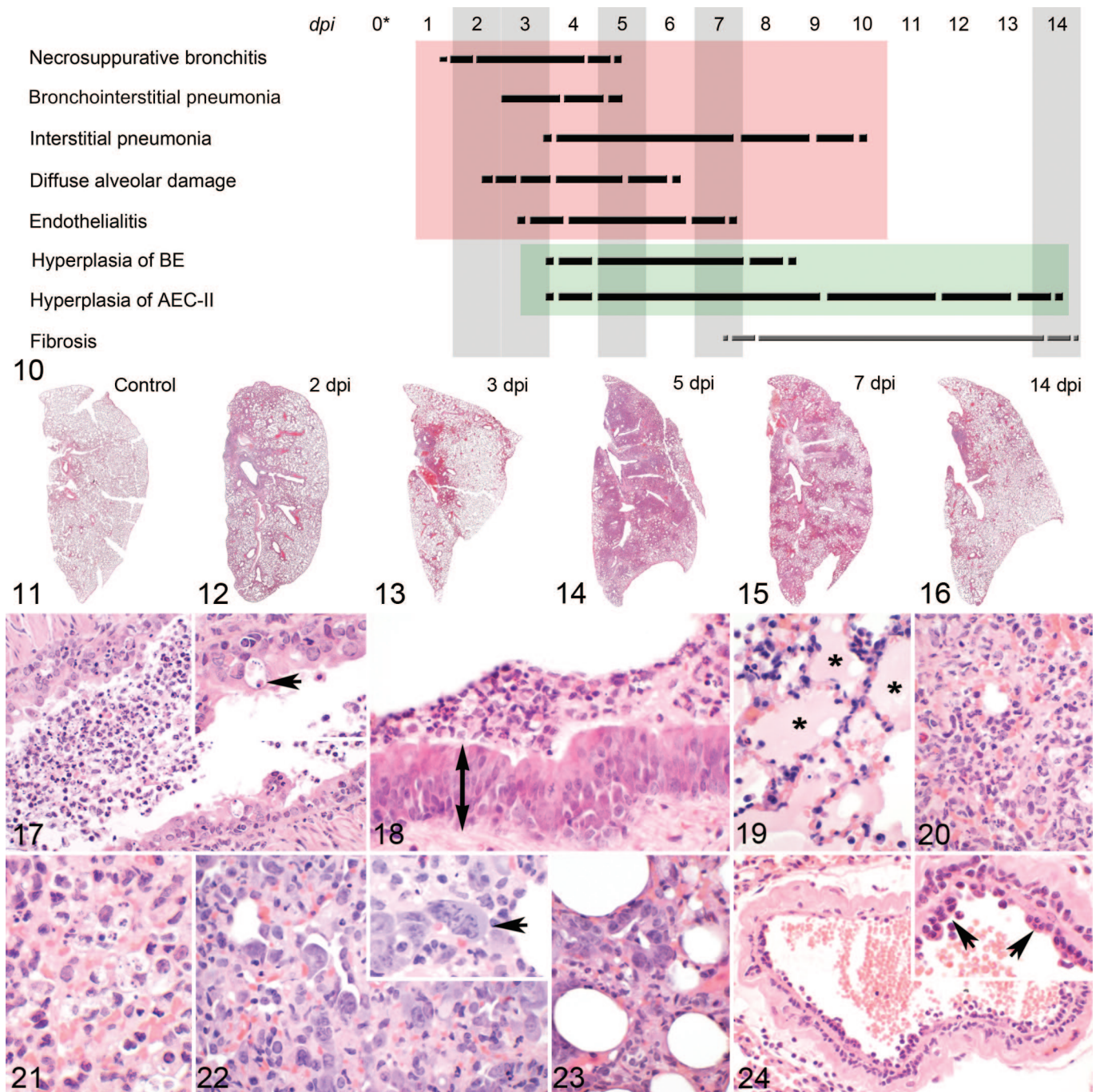
dependent spectrum in severity. Marked pneumomediastinum was observed in all animals at 4 to 6 dpi and interpreted as secondary to micropulmonary rupture. At 20 dpi, only minimal residual lung abnormalities were detected by micro-CT.

At necropsy at 3 or 5 dpi, gross lesions of the lungs included patchy to diffuse consolidation with atelectasis, edema, and failure to collapse after removal, all consistent with interstitial pneumonia.⁷⁰ No substantial lesions have been reported for other tissues so far.

Histopathology

Virtually all reports on experimental infections in Syrian hamsters include histopathological observations starting at 2 dpi, and consistently describe various patterns of virus-induced cell and tissue damage accompanied by a self-limiting inflammation throughout the entire airways, almost completely resolving around 10 to 14 dpi (Figs. 10–24).^{13,23,44,61,70,77} Dose-dependent differences when using between 10³ and 10⁵ pfu were seen in the speed of progression but less in lesion severity.^{44,70} A single study reported systemic extrapulmonary spread of the virus and multi-organ disease after high-dose infection using 10⁶ pfu.⁸⁰ Several publications, however, offer only fragmentary information on procedures and descriptions of lesions, highlight different time points after infection, and partly use inconsistent and diverging terms to describe obviously similar lesions. Systematic comparisons of different reports thus appear difficult. Based on all available descriptions and a systematic comparison with lesions considered relevant in COVID-19 patients, a standardized nomenclature was therefore proposed with guideline character for histopathology reports on SARS-CoV-2-induced pneumonia in hamsters.³⁹ The following overview will adhere to these recommendations. For more general background on common reaction patterns in airways and lungs, the reader is referred to excellent reviews on respiratory tract pathology of mice and rats.^{28,65}

The first lesions observed after transnasal inoculation of the virus affect the upper respiratory epithelium, particularly of the nasal conchae. Virus-induced cell swelling, loss of cilia, and finally necrosis with desquamation and evidence of apoptosis at 2 to 4 dpi are accompanied by blood vessel congestion and patchy to diffuse intra- and subepithelial infiltration with monomorphonuclear cells and neutrophils.^{23,70} Massive damage to and desquamation of the OE starts at 2 dpi with loss of cilia and subsequent infiltration with immune cells in the OE and the lamina propria.^{18,25} As early as 2 dpi, necrosuppurative tracheitis, bronchitis, and bronchiolitis are consistently observed with luminal influx of neutrophils and macrophages, along with epithelial cellular debris (Figs. 10, 17, 18). From 3 to 5 dpi, the primarily perihilar lesions progress centrifugally toward the lung periphery with a patchy, bronchointerstitial pattern from large bronchi down to the terminal bronchioles with strong luminal, intraalveolar, and interstitial influx of neutrophils and macrophages, along with marked perivascular lymphocytic cuffing. Only a single group reported that the right cranial lung lobe was most commonly affected first and that the



Figures 10–24. SARS-CoV-2 infection, lung, Syrian hamster. **Figure 10.** Characteristic lesions can be divided into a phase of tissue damage and inflammation (red box) followed by regeneration (green box). Fibrosis is not a consistently reported feature in this model. Gray boxes show the time points for which histological analyses are available, and the remaining time points represent estimates. Asterisk, infection with SARS-CoV-2. BE, bronchial epithelial cells. AEC-II, alveolar epithelial type II cells. **Figures 11–16.** Overview of time-dependent microscopic lesions of the entire left lung (hematoxylin and eosin, HE). **Figure 11.** Uninfected control, 3 days after transnasal application of 30 μ l of Dulbecco's Modified Eagle Medium (DMEM). **Figures 12, 13.** Early bronchointerstitial pneumonia at 2 and 3 days post infection (dpi) begins to spread into alveoli surrounding the hilus. **Figure 14.** Severe diffuse interstitial pneumonia peaks at 5 dpi with little residual bronchitis. **Figure 15.** At 7 dpi, lesions regress with a patchy distribution over the entire lobe. **Figure 16.** After 14 days, almost complete recovery is visible with only little interstitial pneumonia left in a small proportion of hamsters. **Figure 17.** At 3 dpi, bronchi have epithelial necrosis with intraluminal neutrophils and cellular debris. Inset: Necrosis (arrow) of a BE cell. HE. **Figure 18.** Hyperplasia of BE at 5 dpi (double-headed arrow). HE. **Figure 19.** Early infiltration of neutrophils in interalveolar septa, with alveolar edema (asterisks) at 2 dpi. HE. **Figure 20.** At 3 dpi, interalveolar septa and alveolar spaces are densely infiltrated by macrophages and neutrophils. HE. **Figure 21.** Multifocal necrosis of alveolar epithelial cells at 3 dpi. HE. **Figure 22.** At 5 dpi, AEC-II are hyperplastic with occasional bizarre multinucleated syncytia (inset, arrow). HE. **Figure 23.** At 7 dpi, most alveoli are cleared but marked interstitial pneumonia remains. HE. **Figure 24.** At 5 dpi, endothelialitis is present in a medium-sized blood vessel. Inset: endothelialitis, with patches of elevated endothelial cells (arrows) and subendothelial infiltration with mostly mononuclear cells but without thrombosis.

right lung lobes were generally more affected compared to left.⁴⁴ Diffuse alveolar damage (DAD) is milder and has a more patchy distribution compared with reports on human COVID-19.^{11,48} In Syrian hamsters, it peaks around 3 dpi with death and desquamation of AEC, alveolar edema and strands of fibrin admixed with cellular debris, occasional alveolar hemorrhage, alveolar edema, and interstitial as well as intraalveolar influx of neutrophils and macrophages (Figs. 19–21). Hyaline membranes, which are a hallmark of DAD in humans and regularly seen in COVID-19 patients, are virtually not observed in hamsters, with only few controversial reports.^{11,20,23,39,48,80} Heterophils are observed mostly perivascularly in the interstitium where they peak around 5 dpi when the number of neutrophils declines.

Also peaking at 5 dpi, the bronchial epithelium develops marked regenerative hyperplasia with multifocal multilayered, irregularly arranged projections into the lumen (Fig. 18). Due to the multilayered appearance of the bronchial epithelium, some authors refer to this pattern as squamous metaplasia.⁷⁰ Hyperplastic bronchial epithelial cells and AEC-II strongly express the marker of proliferation Ki67.²³ Strikingly similar to COVID-19 patients, large, bizarre, multinucleated cells appear in variable numbers in the alveolar lining which result from fusion and syncytia formation of SARS-CoV-2-infected AEC-II that express the viral S-protein, the cellular receptor ACE2, and the proteases required for receptor docking on their surface (Figs. 22, 25).¹⁴ However, despite this seemingly accepted pathogenesis of these structures, ISH and IHC have failed to localize the virus in these cells in hamsters so far.

Diffuse regenerative hyperplasia of bronchial and alveolar epithelium peaks around 5 to 7 dpi with a cobblestone-like pattern of AEC, drastically narrowing the alveolar spaces, with reduced numbers of neutrophils and macrophages (Fig. 22). At 7 dpi, alveolar spaces are mostly cleared but marked interstitial pneumonia with mostly monomorphonuclear cells and lesser neutrophils in the markedly widened interalveolar septa remains (Fig. 23). Fibrosis is barely seen, with only 2 reports mentioning moderate interalveolar septal and pleural fibrosis at late stages of the disease.^{70,80} Virtually all histological lesions have resolved by approximately 14 dpi in most hamsters with only very little interstitial pneumonia left in single animals.

Of particular interest for the pathogenesis of human COVID-19 is a specific type of vasculitis termed endothelialitis.³ This pattern is seen in SARS-CoV-2-infected Syrian hamsters between 2 and 7 dpi, peaking around 5 dpi. Mostly medium-sized arterial and venous blood vessels exhibit endothelial cell swelling, vacuolation, necrosis, and detachment with subendothelial aggregates of monomorphonuclear cells and rare neutrophils, markedly elevating the endothelium into the lumen (Fig. 24).^{5,39,61,70} Some of these lesions are associated with transmural immune cell extravasation but substantial structural damage to the tunica media or the formation of thrombi have not been observed. Other vascular lesions such as microvascular thrombosis or angiogenesis as seen in human COVID-19 patients obviously do not occur in Syrian hamsters.^{3,11,39}

In the intestine, virus-infected enterocytes were associated with increased monomorphonuclear immune cells in the lamina propria and enlargement of the aggregated lymphoid nodules (Peyer's patches) with activated germinal centers.^{23,80} Depletion of the white and red pulp was reported in the spleen at 2 and 4 dpi, followed by white and red pulp hyperplasia at 14 dpi. Bronchial and mesenteric lymph nodes showed only mild signs of activation.²³

In a single study in which Syrian hamsters were infected with a higher dose of SARS-CoV-2 (10^6 pfu), multisystemic spread of the virus was associated with histological lesions in several other organs.⁸⁰ Red and white pulp of the spleen were depleted with reduced sizes and numbers of lymphoid follicles at 5 and 7 dpi, along with partly phagocytized debris in sinuses. Similar lesions were present in lymph nodes associated with the respiratory tract. In addition, foci of acute coagulative necrosis were observed in the renal cortical tubular epithelium, myocardium, and liver with little or no inflammatory responses. Scattered lymphocytic and neutrophilic infiltrations were seen in the cortex and medulla of the adrenal glands, colocalizing with virus-positive cells of the parenchyma. Focal lymphocytic and neutrophilic inflammation was also observed in the prostate and vesicular glands of male hamsters, along with SARS-CoV-2-expressing cells. No lesions were reported in other tissues including the brains of the hamsters, even after infection with this high dose.⁸⁰

Effects of Age and Sex

Advanced age is a striking risk factor for COVID-19 patients for developing a more severe course of the disease and a lethal outcome than seen in young adults or children.^{95,101} In fact, 2 studies confirmed age-dependent differences in several parameters following SARS-CoV-2 infection in Syrian hamsters. As the 2 studies revealed slightly different results, they will be summarized separately here.

In a first study, 40- to 80-week-old Syrian hamsters showed much more severe clinical signs such as sneezing, shortness of breath, breathing rates, shivering, and lethargy when compared to 7- to 9-week-old hamsters infected under the same conditions.⁷⁶ In addition, a prolonged period of active virus replication was found in the noses of the older animals. Moreover, and in sharp contrast to young animals, more than half of the aged hamsters succumbed to the infection between 5 and 8 dpi. One 80-week-old hamster died of the infection with additional histological evidence of myocardial disease. However, this and all other aged hamsters failed to show any evidence of virus-associated histopathological changes in other organ systems, including the brain.

In a second study, middle-aged Syrian hamsters of 32 to 34 weeks of age exhibited more pronounced weight loss, delayed spread of the virus throughout the lungs, and delayed virus clearance when compared with 6-week-old hamsters.⁶¹ Inflammatory changes were also delayed and less severe, and were interpreted as less effective in clearing of the virus. Nevertheless, alveolar and perivascular edema indicative of

vascular leakage around 3 dpi were stronger in the older animals. Age-dependent differences in endothelialitis were not observed. Importantly, older hamsters still had active tissue damage and interstitial pneumonia throughout the lungs at 14 dpi, when young hamsters had almost completely recovered from all of their lung lesions. Finally, the older hamsters developed lower titers of neutralizing antibodies in their serum than young hamsters,⁶¹ which is in contrast to people where lower titers are commonly reported in younger compared with elderly patients.²¹ However, this difference may be due to the rather early sampling time point at 14 dpi in hamsters while humans are usually tested at later time points after infection.

A second determinant of the clinical course in human COVID-19 is the sex of the patients, with males generally developing more severe outcomes.¹⁰¹ Consistently, male Syrian hamsters of 8 to 10 weeks of age experienced greater morbidity, lost more body weight, developed more extensive pneumonia, and recovered more slowly than females.²⁷ However, no differences were observed in virus replication throughout the respiratory tract. Nevertheless, female hamsters mounted 2-fold higher antibody titers against the virus both in the plasma and lung tissues during the recovery phase.

Thus, the replication of age and sex differences in Syrian hamsters as observed in COVID-19 patients may also allow for mechanistic studies of the disease.⁴⁷

Cellular and Molecular Pathogenesis in the Syrian Hamster

The elucidation of disease mechanisms is of particular interest for the development of targeted therapies and vaccines for humans. It is therefore critical to understand similarities and differences in the pathogenesis of SARS-CoV-2-induced pulmonary, vascular, and immunological changes between hamsters and humans, particularly in light of the established discrepancies in their clinical courses, lesions, and disease outcomes. First studies are available that aimed to elucidate specific disease mechanisms in hamsters or to establish a comprehensive descriptive landscape of relevant cell type-specific and time-dependent molecular pathways.

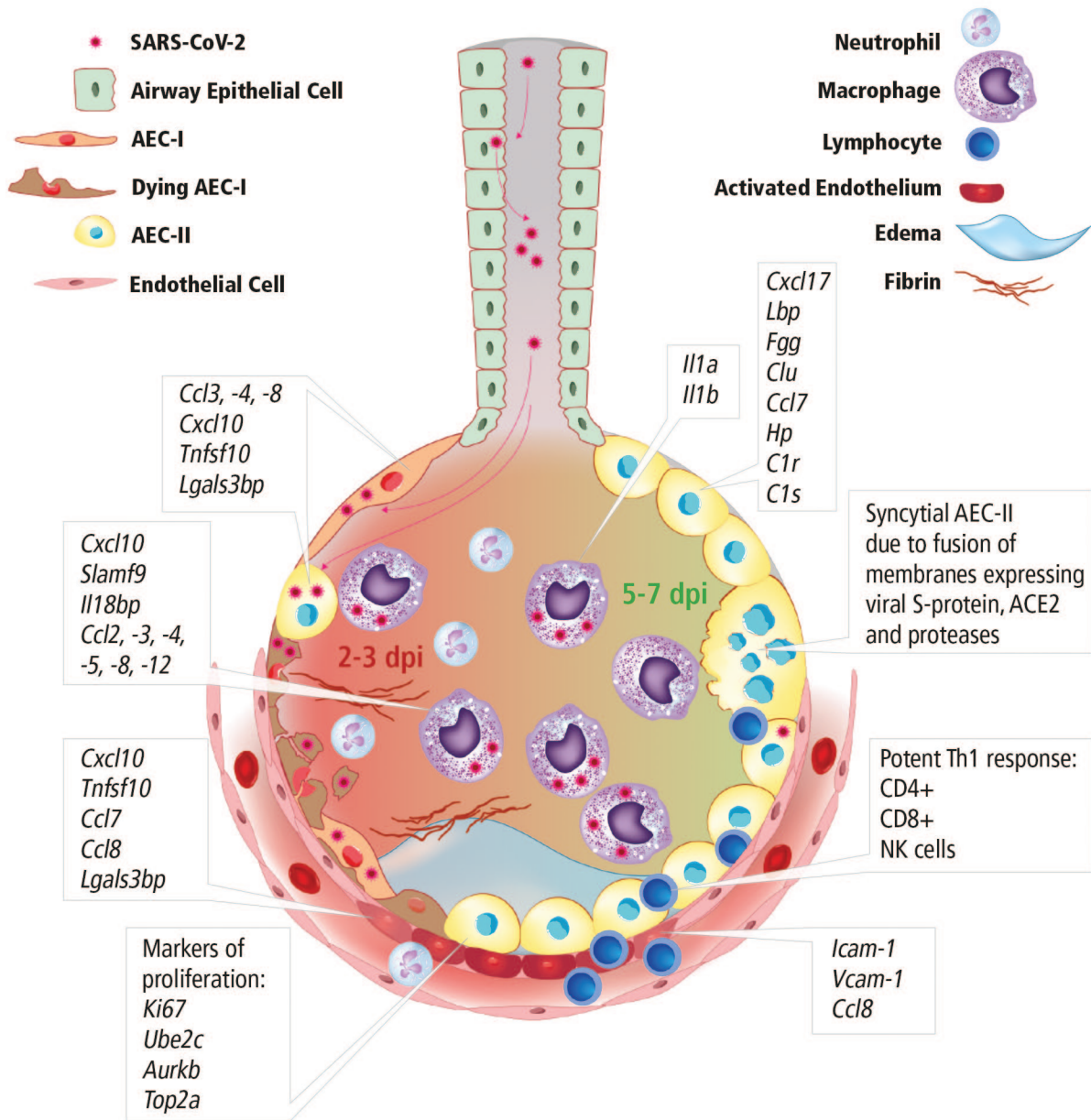
In a broad perspective, infection of hamster upper airway epithelia cells, BE cells, AEC-I, and AEC-II leads to replication and cyclic spread of the virus causing necrotic and apoptotic cell damage to the epithelial lining, which, together with activation and dysfunction but not infection of vascular endothelial cells, results in mild diffuse alveolar damage (DAD) by 2 to 3 dpi (Fig. 25). DAD with alveolar cell debris, edema, and exudation of fibrin as well as other proteins impedes gas exchange and is the pathophysiological correlate of the clinical syndrome of acute respiratory distress syndrome (ARDS), a typical feature in COVID-19 patients with lethal outcome.^{11,20,48}

Decisive for the course of the disease appears to be a rapid, effective, and balanced concomitant activation of a spectrum of immune mechanisms and circulatory changes. Coronaviruses are, like other RNA viruses, typically recognized by cytosolic and endosomal sensors of viral RNA such as RIG-I, Toll-like

receptor 3 (TLR3), TLR7, and TLR8, resulting in activation of NF- κ B and interferons leading to increased expression of pro-inflammatory cytokines and chemokines.^{21,89} Specifically in Syrian hamsters, SARS-CoV-2 infection induces an exuberant signal transducer and activator of transcription factor 2 (STAT2)-dependent type I and III interferon (IFN) signaling, which plays a dual role in restricting infection and dissemination of the virus on one hand but also in driving the development of severe lung disease on the other. The latter is crucial for the pathogenesis and includes overshooting pyroptotic macrophage responses and momentous circulatory changes leading to pulmonary alveolar edema.¹³ As a consequence of activated gene regulation, expressional induction and release of interleukin (IL)-6, IL-10, and IFN- γ , along with the chemokine CXCL-10 as downstream effectors of IFN- γ , lead to the paracrine activation and/or recruitment of leukocytes. This also includes the activation of vascular endothelial cells, which in turn upregulate intercellular adhesion molecule-1 (ICAM-1, CD54) and vascular cell adhesion molecule-1 (VCAM-1, CD106; Fig. 25). Many other facets of early immune defense mechanisms that have been firmly established in other viral infections in humans and mice have not yet been specifically investigated in hamsters but can be expected to be involved. These include the release of danger associated molecular patterns (DAMPs) or alarmins from damaged BE and AEC that further activate neighboring epithelial and endothelial cells as well as leukocytes.⁷³

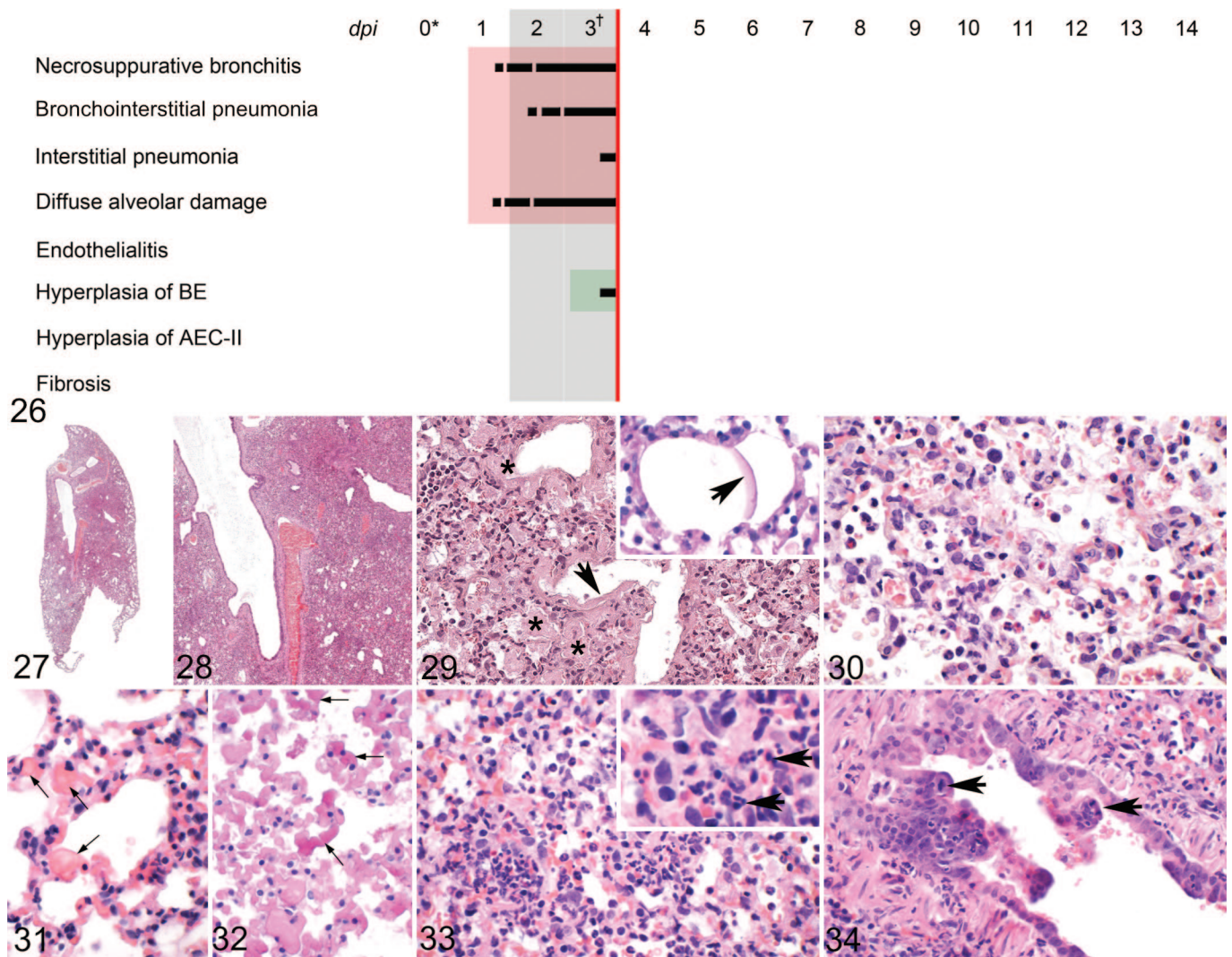
The ignition of strong inflammatory responses immediately after entry of the virus results in a plethora of cellular and molecular cascades that have already been characterized specifically in Syrian hamsters. Cell type-specific whole transcriptome and bulk proteome analyses at various time points after infection have identified a wide spectrum of expressional regulations of genes involved in the orchestration of early (innate) and late (specific) immune mechanisms and cross talks between parenchymal and stromal cells, specifically vascular endothelial cells, and resident as well as infiltrating leukocytes (Fig. 25).⁵⁹

The method used in that study also allowed quantification of the different cell types involved between 2 and 14 dpi, including subsets of alveolar, interstitial, and monocytic macrophages, *Trem14*⁺-monocytes, neutrophils, dendritic cells, B and T lymphocytes as well as natural killer (NK) cells, and resident cell types, including AEC-I, AEC-II, ciliated epithelial cells, endothelial cells, smooth muscle cells, and fibroblasts.⁵⁹ Consistent with histology data, the dominating leukocytes recruited into the alveolus until 3 dpi are neutrophils and macrophages. Around 5 dpi, activated proliferating AEC-II start to dominate and replace and later replenish AEC-I. Also starting around 5 dpi, many alveolar spaces are infiltrated by monocyte-derived macrophages, which contain the highest numbers of virus particles among all other cell types.⁵⁹ They act as prime drivers of virus elimination and regulators of the different immune responses of adjacent cells, while AEC-II display weaker and later transcriptional changes in the orchestration of antiviral defense.⁵⁹



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Figure 25. Cellular and molecular pathogenesis in the alveolus with a selection of relevant immune mediators and other activated genes as detected by temporal and cell type-specific multi-omics analyses from lungs of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-infected Syrian hamsters at 2 to 3 days (left half, red background) and 5 to 7 days post infection (dpi; right half, green background).⁵⁹ Initially, the virus spreads through the upper airways where it infects and multiplies in airway epithelial cells. After entering the alveolar space, it infects both alveolar epithelial type-I (AEC-I) and type-II cells (AEC-II) with effective replication more likely in AEC-II. At 2 and 3 dpi, both types of AEC synthesize and secrete primarily chemokines such as CCL3 and CXCL10 as well as other cytokines that attract neutrophils and macrophages into the interalveolar wall and the alveolar space and activate them. Activated and dysfunctional endothelial and myeloid cells as well as necrosis of AEC-I and also some AEC-II result in leakage of the alveolar wall with exudation of protein-rich edema, together resulting in diffuse alveolar damage. Adjacent vascular endothelial cells are also activated by immune mediators from AEC and macrophages and upregulate, among other factors, vascular adhesion molecules such as ICAM-1 and VCAM-1 that attract leukocytes to the alveolar wall and promote their diapedesis. CD8+ lymphocytes are most effective in eliminating SARS-CoV-2-infected AEC-II. At 5 to 7 dpi, macrophages in the alveolar space carry the highest virus burden, likely due to their phagocytic activity. Proliferating AEC-II result in a cobblestone-like appearance of the alveolar lining and together with restored endothelial cells terminate the leakage of the alveolar wall. Concomitant expression of the viral spike protein, its receptor ACE2, and the required proteases result in fusion of adjacent AEC-II, forming large, bizarre, multinucleated syncytia. Proliferating AEC-II differentiate into AEC-I during the subsequent days and largely restore the structure of the alveolar lining until 14 dpi. A selection of upregulated immune mediators, mostly chemokines and other cytokines as revealed by multi-omics analyses,⁵⁹ are given in white boxes for the different cell types and time points. Gene symbols in italics follow the nomenclature of the murine genome.



Figures 26–34. SARS-CoV-2 infection with the standard dose of 10^5 plaque forming units, lung, Roborovski dwarf hamster. **Figure 26.** Roborovski dwarf hamsters developed drastic bronchial, alveolar, and interstitial lesions at 2 and 3 days post infection (dpi). At 3 dpi, they reached humane end points of the experimental protocol and were euthanized (compare with Fig. 10). Red box, phase of tissue damage and inflammation; green box, phase of regeneration; gray box, time points for which histological analyses are available; the remaining time points represent estimates. Asterisk, infection with SARS-CoV-2. †, death. BE, bronchial epithelial cells. AEC-II, alveolar epithelial type II cells. **Figure 27.** Overview of left lung lobe at 3 dpi with almost diffuse consolidation. Hematoxylin and eosin stain (HE). **Figure 28.** Marked bronchointerstitial pneumonia with additional consolidation of the alveolar parenchyma at 3 dpi. HE. **Figure 29.** Marked alveolar edema and fibrin (asterisks) with formation of hyaline membranes (arrows) at 3 dpi. HE. Inset: Periodic acid–Schiff reaction (PAS). **Figure 30.** Diffuse alveolar damage including necrosis of AEC-I, fibrin deposition, and alveolar edema at 3 dpi. HE. **Figure 31.** Hyaline thrombi in pulmonary capillaries (small arrows) at 3 dpi. HE. **Figure 32.** Hyaline thrombi stain positive with the PAS reaction (small arrows). **Figure 33.** Interalveolar septa and alveolar spaces are densely infiltrated by macrophages and neutrophils with necrosis of AEC (inset, arrows) at 3 dpi. HE. **Figure 34.** Also at 3 dpi, there is some necrosis and flattening of BE with simultaneous mild and patchy hyperplasia with increased mitotic activity (arrows). HE.

Important for the pathogenesis and outcome of the infection, several principally protective measures may also cause collateral damage to host cells or reduce the efficacy of gas exchange, some of which have specifically been demonstrated in lungs of SARS-CoV-2-infected Syrian hamsters. Such mechanisms include, for example, apoptotic death of infected BE, AEC-I and AEC-II, cellular senescence, as well as exuberant alveolar edema.^{6,23,53} In addition, an imbalance between neutrophil extracellular trap (NET) formation (NETosis) and

degradation plays a central role in the pathophysiology of inflammation, coagulopathy, organ damage, and (immuno-) thrombosis in COVID-19 patients,^{2,10} with evidence of similarly dysregulated NETosis in Syrian hamsters.⁵³

Also similar to human COVID-19, hamster vascular endothelial cells react, depending on cell subtypes, by strong and early expression of antiviral, pro-inflammatory, and T cell-recruiting genes without being infected themselves.⁵⁹ The relevance of adaptive T- and B-lymphocytes early in the disease

was shown in a study using recombination activating gene 2 (*Rag2*)-deficient Syrian hamsters that developed aggravated clinical disease and lesions as early as 2 dpi when compared with wild-type hamsters.¹⁶ Between 5 and 7 dpi, recruitment of CD4+, CD8+ cytotoxic, and NK cells by activated endothelia and macrophages coincides with clearance of the virus from infected epithelial cells (Fig. 25). CD8+ cytotoxic and NK cells primarily induce apoptosis of virus-infected AEC. As a hallmark of regeneration peaking around 7 dpi, alveoli are lined by massively hyperplastic AEC-II, which gradually decrease in number by differentiating into AEC-I until 14 dpi. Again consistent with histology findings, cell type-resolved transcriptome and bulk proteome data suggest that virtually all inflammatory responses are resolved by 14 dpi.⁵⁹ Virus-neutralizing IgG and IgM antibodies are detectable in the serum of SARS-CoV-2-infected Syrian hamsters starting around 5 dpi and reach relatively high mean titers of $\geq 1:427$ at 14 dpi, conferring protective immunity.^{23,44,61} The half-life of antibody titers and the duration of protective immunity, however, have not yet been determined.

A systematic comparison of the molecular pathogenesis on the single cell mRNA transcriptome and proteome levels between Syrian hamsters and COVID-19 patients revealed substantial overlaps in their immunoregulatory responses, with particularly striking similarities in their initial pro-inflammatory cytokine surges. On the other hand, a few discrepancies were observed, some of which correlate well with differences from the microscopic perspective. One example is the specific response pattern of monocytic macrophages, which in hamsters displayed a pro-inflammatory expression profile that was productive rather than dysregulated. More specifically, it tended more toward effector T cell-recruiting chemokines targeting CXCR3 and CCR5, and less toward pro-inflammatory cytokine expression that is thought to be responsible for the overshooting, cytokine storm-like responses typical of COVID-19 patients with lethal outcome.^{36,59,102} This variance clearly mirrors the moderate, self-limiting disease seen in young Syrian hamsters, which stands in sharp contrast to COVID-19 patients with lethal outcome.

A further discrepancy to humans became obvious with regard to a lack of activation of transforming growth factor- β (TGF- β). TGF- β is responsible for pulmonary fibrosis as one of the long-term complications in COVID-19 patients but has not been detected in Syrian hamster lungs until 14 dpi, consistent with histological findings according to which fibrosis does not occur at relevant quantities in this model.^{59,70,80,84} More details on the immunopathogenesis of SARS-CoV-2 infection in hamsters have been reviewed elsewhere, including systematic comparisons to humans and other animal models.⁸⁹

Effects of Different Strains and Variants of SARS-CoV-2

The majority of experimental hamster infections have used field isolates of the virus from the early months of the pandemic and from different areas around the globe.^{44,61} Different

effects among the separate isolates have not been reported so far but systematic studies are lacking. During the pandemic, the virus seems to adapt to its new host and to infection- or vaccine-induced immunity. Consequently, several spontaneously mutated variants of concern (VoC) of SARS-CoV-2 attracted much attention due to their alleged or proven increase in virus shedding, transmissibility, virulence, or escape from immune protection elicited by an infection with the original virus or early vaccines.

Several recent studies have employed the Syrian hamster infection model to test for differences between and among various early virus isolates and a number of α , β , δ , or other VoC, respectively, with overall little differences.^{1,60,96} Further studies along this line will likely be published soon.

Divergent Infection Outcomes in Other Hamster Species

Although distinct inbred and outbred strains of Syrian hamsters including the MHA, PD4, LSH, and CB strains have long been established to respond quite differently to other experimental infections,³⁷ such hamster strains have so far not been compared to each other in their responses to SARS-CoV-2 infection. However, drastically different outcomes were observed when other hamster species were used as models, which are all naturally susceptible to the infection due to their similarly high homologies to relevant parts of the human ACE2 receptor.

Of the approximately 20 hamster species known to date, 4 species other than the Syrian hamster have so far been experimentally infected with SARS-CoV-2 and systematically examined. Due to its history in biomedical research since 1957 with more diagnostic tools and molecular information available, the Chinese hamster (*Cricetulus griseus*) was chosen as well as the 3 Phodopus dwarf hamster species—Roborovski dwarf hamster (*P. roborovskii*), Campbell's dwarf hamster (*P. campbelli*), and Djungarian dwarf hamster (*P. sungorus*)—the latter three mostly for their small size and other practical reasons.

The Chinese hamster reproduced viral replication, respiratory lesions, and complete recovery similar to the Syrian, however, with more pronounced and longer clinical signs that allowed better clinical monitoring during the course of the infection.⁸ Lung lesions were slightly milder but more prolonged with DAD and persistence of viral RNA still detectable at 14 dpi. The spectrum of SARS-CoV-2-infected cells was virtually identical to that of Syrian hamsters. Overall, the Chinese hamster model was rated more advantageous compared with the latter, also due to its smaller size, well-characterized genome, transcriptome and translome data, and availability of molecular tools (Table 1).⁸

A drastically divergent course was found when Roborovski dwarf hamsters were infected with the same dose of the same SARS-CoV-2 isolate. A rapid and dramatic drop in body temperature was recorded down to approximately 30 °C at 3 dpi, accompanied by a drastic loss of body weight of up to 20%.⁸⁷ Clinical signs were severe and included snuffling, dyspnea, and ruffled fur with strong reduction of their otherwise quite active

Table 1. Different hamster species develop distinct courses after SARS-CoV-2 infection with different suggested applications as models for human COVID-19.

Model		Course after SARS-CoV-2 infection	Suggested application
Syrian or golden hamster (<i>Mesocricetus auratus</i>)	Young ^{23,44,70,77}	Moderate disease with complete resolution before 14 dpi	Model for moderate, therapeutically less relevant courses of COVID-19
	Aged ^{61,70}	Prolonged disease with delayed virus clearance, complete resolution	Model for elderly or otherwise predisposed COVID-19 patients
Roborovski dwarf hamster (<i>Phodopus roborovskii</i>)	Standard virus dose ⁸⁷	Rapid devastating disease with massive DAD, DIC, and hyperinflammation	Model for highly susceptible patients with elevated risk of lethal outcome
Chinese hamster (<i>Cricetulus griseus</i>) ⁸ Djungarian dwarf hamster (<i>Phodopus sungorus</i>) ⁸ Campbell's dwarf hamster (<i>Phodopus campbelli</i>) ⁸	1/20 of standard dose ⁸⁷	Similar to young Syrian hamsters	} Not yet proposed
		Similar to young Syrian hamsters albeit with more severe and prolonged clinical signs	
		Less susceptible with only mild bronchitis with rapid recovery	
		Least susceptible with only mild bronchitis with rapid recovery	

Abbreviations: COVID-19, coronavirus disease 2019; dpi, days post infection; DAD, diffuse alveolar damage; DIC, disseminated intravascular coagulopathy.

behavior. Three days after infection, multiple individuals appeared terminally ill and had to be euthanized for humane reasons. Histologically, lungs showed massive and highly destructive changes diffusely throughout the entire lungs with DAD including severe AEC necrosis, hyaline membrane formation, proteinaceous material, hemorrhages, edema, and cellular debris within alveoli as early as 2 dpi (Figs. 26–34). Particularly the severe DAD was much more prominent and more widely distributed when compared with the Syrian hamster model, and as such more similar to what is seen in COVID-19 patients.^{11,48} Also in contrast to Syrian hamsters, hyaline thrombi were massively present in interalveolar capillaries (Fig. 31), strongly staining with the periodic acid Schiff (PAS) reaction (Fig. 32), indicative of disseminated intravascular coagulopathy (DIC) and hyperinflammation.³⁶ On the other hand, bronchitis was rather mild (Fig. 34) and endothelialitis as well as pronounced hyperplasia of BE or AEC were not observed,⁸⁷ possibly due to their deteriorating course early after infection.

Interestingly, when Roborovski dwarf hamsters were infected with only 1/20 of the initial dosage (ie, 5000 pfu) of the same SARS-CoV-2 isolate, they almost completely recapitulated the disease as seen in Syrian hamsters infected with the high dose, including endothelialitis, epithelial cell hyperplasia, and no hyaline membranes or evidence of DIC. It was thus hypothesized that this dwarf hamster species is much more susceptible to the virus with an otherwise similar repertoire of clinical, immunological, and pathological responses when compared with the Syrian albeit at a much lower dosage of the virus. On the other hand, the standard/high-dose infection (10⁵ pfu) model of the Roborovski dwarf hamster may be helpful to better model the course of COVID-19 as seen in highly susceptible, mostly elderly patients, which are generally much more prone to developing severe disease with lethal outcome.⁸⁷

The molecular pathogenesis in the Roborovski dwarf hamster model has not yet been established but a time-resolved scRNASeq-based transcriptomic landscaping approach as recently performed for the Syrian hamster is on its way

(Emanuel Wyler, personal communication).⁵⁹ Interestingly, virus-induced senescence seems to be involved which may offer novel treatment options.⁵³ It will be interesting to explore whether hallmarks of COVID-19 in susceptible patients with lethal outcome can be identified in this model, such as hyperinflammation, cytokine surge, dysfunctional vascular endothelia, and dysregulation of the coagulation system with micro-(immuno-) thrombosis and microvascular hyperpermeability due to CD8+ cytotoxic T-cells attacking endothelial cells and leading to vascular barrier destabilization.^{10,36,102}

When the 2 other dwarf hamster species, Campbell's and Djungarian dwarf hamsters, were infected with 10⁵ pfu of the same virus isolate, they both developed clinical and pathological responses similar to or even milder than those of the Syrian and Chinese hamsters, and also milder than those of the Roborovski dwarf hamsters infected with the low dosage. Both largely recovered during the first week after infection, with the Campbell's dwarf hamster seeming least susceptible among all species tested so far. Strong regenerative hyperplasia of BE and AEC started at 3 dpi, without evidence of appreciable DAD, hyaline membranes, endothelialitis, or DIC. It was thus concluded that these 2 dwarf hamster species would not offer obvious additional value to the already existing models.⁸⁷

Consequently, infection of the Roborovski dwarf hamster at the standard dose of 10⁵ pfu seems like the only naturally susceptible animal model available to date that provides the sensitivity required to develop and test the efficacy and safety aspects of therapies and vaccines for the particularly vulnerable groups of patients in this pandemic, such as the elderly, immunocompromised patients, or patients with predisposing conditions (Table 1).

It appears noteworthy that different closely related animal species may recapitulate different courses of a wide spectrum of disease manifestations as seen in human COVID-19. To our knowledge, this has not yet been described for other model species or any other human disease. In this regard, it is tempting to speculate on possible reasons of the drastically different

outcomes of SARS-CoV-2 infection between the Roborovski dwarf hamster and the other 4 species investigated so far. The natural habitat of the former in Northern China and Mongolia seems to not differ much from the habitats of the other. Little is known about possible evolutionary discrepancies with regard to their natural exposures to pathogens, which may have shaped diverging components of their immune systems. Comparative genomic and transcriptomic studies are under way and may help unravel the mechanisms underlying their different susceptibilities to SARS-CoV-2 infection on the genetic and molecular levels, ultimately helping understand human COVID-19. Caution needs to be warranted, however, in that at least some of the differences reported to date may have resulted from distinct breeding and husbandry conditions. Specifically, the 4 additional hamster species examined so far were all obtained from a non-specified pathogen free (SPF), non-standardized pet breeding facility with likely different microbiomes, infection histories, and other determinants that may have resulted in distinctive primings of their immune responses.^{8,87}

Technical Limitations of Hamster Models

Several limitations apply to hamster models of COVID-19, both with regard to technical readout options and in terms of factors that influence the outcome and reproducibility of experimental infections.

Although Syrian hamsters with several mostly outbred lines have been employed for decades in experimental research, and despite the availability of several knockout lines,^{13,16,37,70} much less diagnostic and molecular tools are at hand when compared with the mouse, including antibodies applicable for IHC. ISH of mRNA molecules is available as an alternative but may yield different information depending on, for example, the expression levels or biological half-life of the protein or mRNA molecules of interest, respectively. As an interesting alternative, much progress has been made in recent years in the fields of spatial proteomics and transcriptomics, which allows the quantification of the individual expression levels of virtually all proteins and mRNA molecules with single cell-type resolution. Fortunately, these have already been applied to characterize the pathology and immune responses in SARS-CoV-2-infected Syrian hamsters,^{59,82} allowing for direct comparisons with similar data derived from human patients.⁵⁹ Clearly, this methodological progress inaugurates a new era in the understanding of infection pathology and immune responses on the molecular level in a cell type- and time-dependent resolution.

A second drawback of hamsters having been used only sporadically in biomedical research during the past decades is the lack of available inbred strains and species bred under standardized, controlled conditions. While currently only a single line of Syrian hamsters is readily available via most commercial breeders of laboratory animals, Chinese hamsters fulfilling laboratory standards such as standardized genetics and SPF status are much more difficult to obtain. Even more serious limitations exist for the Roborovski dwarf hamster, which is bred as popular pet animal in several countries and

practically available only via commercial pet vendors, usually without any genetic or hygienic standards. The lack of SPF status is of particular concern for infection and immune research because unrecognized co-infections with other pathogens, diverse individual infection histories, and variable, uncontrolled microbiomes might drastically affect their responses to experimental infections with specific pathogens. These shortcomings are particularly limiting for aspects of research quality management, such as experimental robustness, interpretation, and reproducibility of results and the standardized reporting of key experimental conditions.^{7,31} Definitely, in any of these factors, the laboratory mouse has and for the foreseeable future will have the lead over hamsters.

An Ethical Perspective on Research Quality Management

Today's limitations in available options for such research quality measures in hamsters as models for humans also touch ethical issues. In general, the use of animals in preclinical research can only be ethically legitimized when minimal criteria of research quality standards are met, helping keep their suffering at an unavoidable level. In addition to the more general 3R principles of *Replacement*, *Reduction*, and *Refinement* as firmly established principles for the ethical use of animals,⁷¹ the PREPARE guideline helps optimize study designs, including appropriate statistical methods to reduce the number of animals required for a particular purpose.⁷⁸ Further criteria that secure the scientific value and the sustainable usability of animal research data are *robustness*, *registration*, and *proper reporting* of both procedures and results, which extend the 3Rs to a 6R principle.^{31,33,63,81} This is particularly imperative in a situation with high pressure toward research output under conditions of a pandemic, with almost 200 research publications on SARS-CoV-2-infected hamsters within the first 12 months.^{31,33,63}

In light of such principles, some of the available reports on hamster infections with SARS-CoV-2 appear incomplete in terms of the reporting of critical experimental information, such as the virus strain, strain and origin of hamsters used, age and sex of animals, or euthanasia procedures that may affect histology of the lungs. Moreover, readout parameters and the choice of lesions and terminology used to describe histological changes vary widely among these reports, which makes cross-study comparisons and meta-analyses more than challenging. Consequently, a standardized list of relevant lesions and terminology to be used in hamsters as models for COVID-19 was proposed, based on established relevant lesions in the human disease.³⁹ Such lists with guideline character had previously been established for general aspects of rat and mouse respiratory tract lesions,⁶⁵ and specifically for mouse models of infectious pneumonia,²⁸ which help resolve the constant tension between academic exploration and quality-oriented confirmation studies in preclinical biomedical research.³³

Table 2. Similarities and principle differences between Syrian or Roborovski dwarf hamsters and humans following SARS-CoV-2 infection. Effects may vary depending on the infectious dose.

<i>Observed in hamsters and COVID-19 patients</i>
1. Similar cellular expression pattern of the virus receptor angiotensin converting enzyme-2 (ACE-2), resulting in similar target cells
2. Ageusia and anosmia with infection of the olfactory epithelium (only described for the Syrian hamster)
3. Early bronchointerstitial pneumonia turning into patchy interstitial pneumonia
4. Diffuse alveolar damage (DAD; more strongly and with hyaline membranes only in the Roborovski dwarf hamster)
5. Bizarre multinucleated giant syncytia formed by alveolar epithelial type II cells
6. Dysregulated NETosis (only described for the Syrian hamster)
7. Vascular endothelialitis in the lungs (Syrian hamster and low dose-infected Roborovski dwarf hamster)
8. Coagulopathy with hyaline thrombi in pulmonary arterioles and capillaries (Roborovski dwarf hamster only)
9. Age and sex dependence of lesion severity (only described for the Syrian hamster)
10. Aggravation of disease by immunocompromising factors
11. Patchy infection of the intestinal epithelium with little if any consequences
<i>Not yet recapitulated in hamsters</i>
1. Asymptomatic courses with silent seroconversion
2. Extrapulmonary multi-organ vascular lesions and thromboses
3. Neuroinvasion and encephalitis
4. Long COVID-19 with pulmonary fibrosis
5. Chronic fatigue syndrome (CFS)

How Useful Can Hamster Models of COVID-19 Be?

Animal models are usually employed when questions cannot be addressed in patients or existing *in vitro* models, but how similar can hamsters be to humans after SARS-CoV-2 infection? Overall, the Syrian and Roborovski dwarf hamster models appear to recapitulate many characteristic albeit not all aspects of the human disease (Table 2).

Importantly, which differences may be decisive for the outcome of SARS-CoV-2 infections in hamsters, specifically in the testing of drugs and vaccines and their inference to humans? Many decades of experiences with mice in infectious disease research have revealed a wide spectrum of relevant species-specific differences, other than simple discrepancies in the structure and binding affinity of a cellular virus receptor such as ACE2. Particularly critical for the outcome of virus infections, the toolbox and mechanisms of the murine immune system possess many differences when compared with humans, including distinct TLRs, defensins, and subsets of leukocytes and antibodies, to name a few.²¹ Similar differences can likely be anticipated in hamsters, the immune system of which, however, is to date far less understood than that of mice. In any case, the practical value of an animal model largely depends on the usefulness of clinical, pathological, virological, and other

readout parameters with their similarities and differences to the human disease.

The general usefulness of any model depends on its being fit-for-purpose,²⁶ so for which purposes can available hamster models of COVID-19 be used? Before we discuss their potential value for separate applications in basic, preclinical, and clinical research, the particular challenges that COVID-19 (beyond those for most other viral diseases) poses to a model need to be outlined. First, unlike hardly any other virus infection, the clinical outcomes vary across a remarkable range from asymptomatic or mild courses over a wide array of diverse organ symptoms to dramatic disease over weeks or months with an elevated risk of multi-organ failure and lethal outcome.^{34,40,64} Is it conceivable at all that any single model could cover this spectrum, which would include the necessity to cover the relevant individual risk factors, including age, sex, and comorbidities, such as cardiovascular disease, hypertension, diabetes mellitus, obesity, and chronic respiratory disease?^{64,101} Second, the clinical courses with highest concern in humans that urgently require animal models to develop improved therapeutic interventions are seemingly caused by imbalances of immune functions, particularly a pro-inflammatory cytokine surge primarily driven by macrophages, and endothelial dysfunction resulting in multi-organ thromboses.^{36,64,88,102} Can a small rodent be expected to mimic such complex dysfunctions, at least some of which are based on interindividual genetic variations in humans?⁴

Understanding Mechanisms of the Disease

There is wide agreement that the clinical, virological, and histopathological changes observed in SARS-CoV-2-infected Syrian hamsters closely mimic core aspects of a moderate course of COVID-19 as typically seen in middle-aged patients without substantial comorbidities.^{23,70,77,101} For this group of patients, mechanisms that result in the spread and clearance of the virus, balanced inflammation, full restoration of pulmonary structure and likely function, as well as protective seroconversion may apparently be studied in the Syrian hamster. Of note, several of the peculiar lesions typically seen in patients are similarly recapitulated in Syrian hamsters, such as vascular endothelialitis and the formation of bizarre, multinucleated AEC-II syncytia, supporting the notion of widely overlapping mechanisms. Still, some uncertainty remains as to whether identical mechanisms actually apply in humans and Syrian hamsters for all of these aspects. Although a first systematic comparison on the single cell transcriptome and proteome levels revealed major overlaps with data from human patients,⁵⁹ conclusions on similarities and differences in the innate and specific immune pathways and molecular mechanisms remain largely speculative today.

On the other hand, extrapulmonary endothelialitis and other vascular lesions that may lead to multi-organ thromboses in severely affected COVID-19 patients have so far not been observed in Syrian hamsters. Moreover, there is little evidence to assume that this model can be used to study long-term

complications that may develop in patients after a mild, acute, and self-limiting course of the disease, such as long COVID-19 with lung fibrosis or chronic fatigue syndrome (CFS).^{9,47,84}

Clearly, the Syrian hamster appears unsuitable to model severe courses of COVID-19 as seen in patients with relevant comorbidities. Theoretically, it is conceivable to first establish hamster models of diabetes, atherosclerosis, or chronic obstructive pulmonary disease, to name a few, and then infect them with SARS-CoV-2 to study the interferences of their pathogenesis. In fact, effects of temporary or permanent, genetically induced immunosuppression on the course of SARS-CoV-2 infection in Syrian hamsters have already been established.¹⁶ The majority of such approaches, however, could likely be pursued more practically in combinations with hACE2-transgenic mice, as suitable models of common human comorbidities are already available and well established in mice.

In sharp contrast to the Syrian, the Roborovski dwarf hamster model appears to well recapitulate the drastic disease outcome as seen in some highly susceptible, mostly elderly patients. Here, the clinical signs and histopathology are strongly indicative of exuberant pro-inflammatory macrophage activation with a cytokine surge resulting in devastating DAD, vascular endothelial dysfunction, and coagulopathy.⁵⁹ Other therapeutically promising aspects such as virus-induced senescence have also been observed in the Roborovski dwarf hamster, like in human COVID-19 patients.⁵³ Still, the comorbidities considered relevant in humans are not involved in this model either. Moreover, the time frame of very few days as observed in the Roborovski dwarf hamster is at variance with drastic courses in human patients that usually take several weeks and likely involve the presence of serum antibodies.^{34,40,64} Thus, despite obvious similarities, substantial differences in the pathogenesis must be anticipated that warrant clarification in the future.

Testing Antiviral Therapies

Substances that target virus replication directly by interference with mechanisms important to virus biology, such as entry to host cells,⁷⁹ RNA replication,⁹⁸ protein synthesis,⁹² or virus egress³⁸ can strongly rely on virological parameters as primary endpoints and are thus assessable in the early phase of SARS-CoV-2 infection in Syrian hamsters. Consequently, numerous preclinical testing of diverse antiviral agents and other measures destined for combatting replication or shedding of the virus in humans have already used this model. These include drugs that directly antagonize viral replication such as remdesivir and clofazimine,^{98,100} as well as antiviral antibodies that target the virus and help inactivate it by the immune system.^{35,49} Moreover, another study successfully examined the efficacy of wearing surgical face masks in the prevention of viral spread among Syrian hamsters, extending the usefulness of this model to epidemiological dimensions.²² However, species-specific differences to humans need to be respected in such studies, particularly the fact that replication of SARS-CoV-2 in hamsters is confined to the first days following

infection with rapid decline after 3 dpi,^{44,61,77} while replication and shedding of the virus in humans usually last much longer.⁷⁴ Despite this and other limitations mentioned earlier, it appears that the Syrian hamster model is largely accepted for the testing of antiviral therapies in the biomedical community at eye level with mouse models of other diseases.

The much higher susceptibility of the Roborovski dwarf hamster to developing drastic disease after SARS-CoV-2 infection likely makes it a much more sensitive model to develop and test antiviral therapies.⁸⁷ In light of its resemblance to highly susceptible human patients, it appears reasonable to assume much stronger predictive value for observations from this model when aiming at protecting or saving the group of patients that causes highest clinical concerns.

Testing Drugs That Target Host Mechanisms

The successful testing of therapeutic interventions that target specific host pathways or individual molecules in any model largely depends on similarities in their targets, both in terms of structure and function as well as their integration into larger functional networks, and the overall pathogenesis of the disease. Although the Syrian and the Roborovski dwarf hamsters share many features with less or highly susceptible patients, respectively,^{53,87} various unknowns and uncertainties with regard to similarities and differences to humans in many aspects of complex disease mechanisms and individual molecules remain. Caution is therefore warranted when targeting possibly species-specific targets in, for example, cellular pathology, innate or specific immunity, inflammation, or regeneration and repair. Recent progress in omics-methods that decipher genomic information and the regulation and interaction of individual molecules and pathways, however, can be expected to facilitate such comparisons in the near future.

On the other extreme, could drugs that act upon mechanisms or molecules with little or virtually no species specificities across mammals be tested in hamsters with robust inference to people? Among these drugs with high evolutionary conservation of their receptors are, for example, glucocorticoids, to which promising properties have been attributed for chronic COVID-19 patients with hyperinflammatory syndrome and vasculitis.^{19,90} Not unexpectedly, beneficial effects have also been observed in Syrian hamsters with alleviated body weight loss as well as improved nasal and pulmonary inflammation following SARS-CoV-2 infection.⁹⁸ Still, as deregulated immune responses or mechanisms leading to hyperinflammation are not usually seen in the Syrian hamster model, the transferability of results to humans remains limited, despite high homologies in their drug receptors. A second compound with inhibitory effects on cytokine production and modulation of certain costimulatory molecules, hydroxychloroquine, proved ineffective in the treatment of SARS-CoV-2 infection in Syrian hamsters.⁶⁹ Here, similar restrictions probably apply in terms of projections to human patients from a model in which an unleashed immune response simply does not occur.

A frequently overlooked restriction in preclinical testing is the pharmacokinetic of target substances. Pharmacokinetic studies are typically not performed in hamsters and results from mice or rats do not always translate well to hamsters. In addition, not all routes of administration are feasible in hamsters. For example, intravenous dosing is much more challenging in hamsters compared to long-tailed rodents such as mice or rats.³²

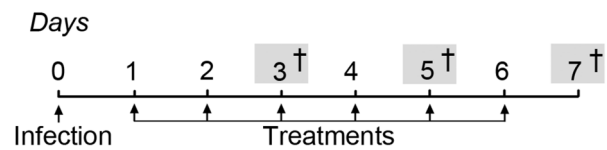
Testing Vaccine Candidates

The availability of effective and safe vaccines with relevant properties such as their costs, required storage conditions, and efficacy against VoC is by far the most effective and sustainable weapon to defeat the pandemic with its globally devastating consequences. Sterilizing immunity, indicated by the absence of virus replication after challenge, is the primary goal in vaccine development.⁴⁵ The quest for an appropriate model that allows testing of vaccine candidates with high predictive value for people has fueled research on hamster models very early in the pandemic.⁹⁴ Absence of virus replication, the primary endpoint of vaccine trials, can be conveniently assessed in hamsters at early time points, typically 2 to 5 dpi.⁹⁷ In fact, a wide spectrum of attenuated,^{46,91} genetically engineered,¹⁵ or vector-based vaccine candidates have been scrutinized in Syrian hamsters.^{51,85} In addition to investigating the principle efficacy and safety of the different candidates, other goals have been pursued such as the verification of their protective effects against spontaneous SARS-CoV-2 variants, including the allegedly more infectious or more virulent VoC strains B.1.1.7 (α) and B.1.351 (β).⁸⁶ However, while vaccine efficacy early after vaccination (typically 3–5 weeks) is easily studied in animal models, observing the duration of vaccine-induced immunity remains challenging not only with respect to the resources required for intensive long-term husbandry but also due to the overall difference in metabolism and life expectancy between rodents and humans.

Typical Assay Workflows and Readout Parameters

In consideration of the rather rapid course of SARS-CoV-2 infection in hamsters, studies that aim at testing therapies and vaccines in this model should likely be designed to capture target readouts at one or multiple time points typically within 1 week following infection (Fig. 35). Sample analysis at the peak of virus replication between 2 and 3 dpi is suggested for therapies and vaccines that target virus replication. However, other important parameters, such as body weight loss or essential effects of lung pathology, may not be pronounced at these early time points. While active virus replication is declining sharply from 5 dpi, assessment of viral RNA offers the possibility to make judgments about antiviral efficacy at time points beyond 5 dpi. Depending on the exact question, sampling time points between 4 and 7 dpi offer possibilities to judge clinical

Typical Therapeutic Experiment



Typical Vaccination Experiment

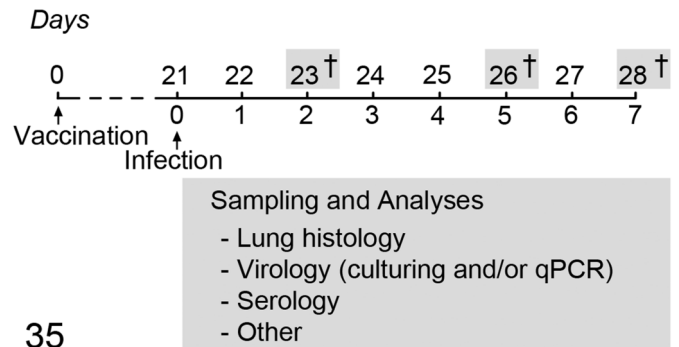


Figure 35. Proposed time lines for typical therapeutic and vaccination experiments in Syrian hamsters with SARS-CoV-2 infection. In therapeutic interventions, the first week following infection is the most useful time frame to observe the clinical outcome and take samples for virological and histological examination. In vaccination experiments, 21 days following vaccination is a typical time point for challenge infection that allows the vaccine to have elicited effective immune responses. The week after challenge infection is a useful time frame to examine clinical efficacy and to sample animals to assess other parameters of vaccination success. †Indicates recommended sampling time points.

efficacy based on weight developments and relevant aspects of lung pathology.

Histopathological analyses of the lungs should be performed under standardized conditions and readout parameters with defined terminology.³⁹ In order to obtain quantitative, statistically testable data for each relevant histology parameter, evaluations can be extended by computed digital image analyses,²⁹ scoring schemes,²⁸ or Cavalieri's principle.³⁰

Lessons Learned From Hamster Models of COVID-19

As COVID-19 will not be the last pandemic requiring urgent establishments of suitable preclinical models, it seems imperative to recapitulate our experiences made with the hamster models at this occasion. Thanks to available genomic sequence information and molecular modeling tools, naturally susceptible animal species were rapidly and correctly predicted,^{17,23,99,101} which may in the future allow the identification of even different species as suitable model hosts for the agent of a next pandemic. For hamsters, it appears like an extraordinary fortuity that

closely related species like the Syrian and Roborovski dwarf hamsters mimic different courses of the diverse human disease, both relevant for mechanistic studies and preclinical testing. Despite their high popularity due to numerous relevant similarities to human COVID-19, both models are less than perfect and possess technical and practical limitations. In any case, much can be learned in retrospect from the early and rapid, almost global establishments of hamster models in the SARS-CoV-2 pandemic, including the indispensability of proper and complete reporting of relevant experimental procedures and standardized readout parameters, among other research quality measures. Just as essential will be the integration of single cell type and time-resolved transcriptome and proteome data,^{59,82} along with established classical histopathology, IHC, and ISH methodologies.^{39,61,70} Regardless of the next model species of choice, comparative veterinary pathology will be prepared and indispensable for the characterization of appropriate models, their similarities and differences to what will be known from the human condition, and for the careful development and testing of effective and safe therapies and vaccine candidates for humans.

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Addendum

Recent evidence from mice with severe alveolar damage due to infection with H1N1 influenza virus or SARS-CoV-2 suggests that the dense cobblestone-like hyperplasia of epithelial cells within alveoli around 5 dpi and later (compare to Figs. 10, 22, 25 in this article) does in fact not represent mere hyperplasia of alveolar epithelial type II cells, AEC-II. Instead, a significant amount of these cells may result from migration and dysplastic differentiation of rare p63+ progenitor cells that reside in major airways and populate the alveolar wall where they express the basal epithelial marker cytokeratin 5 (Krt5). These cells are seemingly not subject to virus infection, thereby enabling coverage and protection of the alveolar wall while the pathogen is cleared from susceptible cells, including AEC-II. Whether this

process, termed *dysplastic alveolar regeneration and remodeling*, also occurs in hamsters remains to be addressed, like several open questions related to this scenario, including the fate of these cells.

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