Genetically diverse mouse models of SARS-CoV-2 infection model clinical variation and cytokine responses in COVID-19.

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Abstract

Host genetics are a significant determinant of coronavirus disease 2019 (COVID-19)¹. Animal models that reflect genetic diversity and a range of clinical outcomes observed in human populations are needed to understand mechanisms of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection dynamics and disease². Here, we report a mouse panel comprising the diverse genetic backgrounds of the Collaborative Cross (CC) founder strains crossed to C57BL/6J mice expressing the K18-hACE2 transgene³ that enables infection by SARS-CoV-2. Infection of CCxK18-hACE2 F1 progeny resulted in a spectrum of weight loss, survival, viral replication kinetics, histopathology, and cytokine profiles, some of which were sex-specific. Importantly, survival was closely associated with early type I interferon expression and a phased proinflammatory response distinct from mice with severe disease. Thus, dynamics of inflammatory responses observed in COVID-19 can be modeled in diverse mice that provide a genetically tractable platform for understanding antiviral immunity and evaluating countermeasures.

Main

Mice humanized for the main cellular receptor for SARS-CoV-2 entry, angiotensin converting enzyme 2 (ACE2), are valuable pre-clinical infection models². The most widely used model, K18-hACE2, supports high early virus replication in lung epithelial cells, resulting in inflammatory cell infiltration, interstitial edema and focal consolidation of the lung with rapid lethality⁴⁻⁹. However, this model on a standard C57BL/6J genetic background does not reflect the wide variety of COVID-19 outcomes in humans, from asymptomatic to severe¹⁰, that are

dependent on genetic polymorphisms, age, sex and the presence of underlying conditions¹¹⁻¹³. Multiple studies have demonstrated the value of using genetically diverse mice to assess the impact of host genetics on infectious disease outcomes¹⁴⁻¹⁸. Indeed, a single genetic background likely will not capture the range of responses and can fail to model key features of disease¹⁹.

To determine if incorporating genetic diversity could recapitulate the broad phenotypic variation observed in human COVID-19, we crossed the K18-hACE2 strain to the genetically distinct inbred strains A/J, 129S1J, NOD, NZO, PWK, CAST, and WSB that along with C57BL/6J comprise the founders of the Collaborative Cross (CC). This reference population represents 90% of the genetic diversity in *M. musculus* populations^{20,21} and are used to understand genetic regulation of complex immune responses. Notably, NOD and NZO mice are commonly used in studies of diabetes and metabolic dysfunction that are factors contributing to COVID-19 severity²². In addition to the 8 CC founder strains, BALB/c and DBA strains were included because BALB/c is widely used in infectious disease modelling, and DBA is a founder strain for BXD strains also used in genetic mapping²³.

CCxK18-hACE2 F1 mice were infected via intranasal inoculation with 10³ pfu SARS-CoV-2 strain nCoV-WA1-2020 (MN985325.1) and mice were monitored for 21 days. This virus dose is sub-uniformly lethal in the prototype K18-hACE2 strain (C57BL/6J background), resulting in 80-90% of mice reaching end-point criteria by 7 days post infection (dpi) (Fig.1). Infection was confirmed by seroconversion to SARS-CoV-2 nucleoprotein in all survivors. Comparative analysis of the eight CCxK18-hACE2 F1 cohorts documented a broad spectrum of weight loss and survival curves characteristic to each CC founder genotype, some of which were sex-specific

(Fig. 1). Highly sensitive mice strains included C57BL/6J and A/JxK18-hACE2 that lost approximately 20% of their starting weight between 4 and 7 dpi with no clear sex bias. In contrast, CCxK18-hACE2 F1 progeny from PWK, NZO, 129S1/J (Fig. 1), BALB/c and DBA (Fig. S1) were resistant to clinical disease, generally losing 5-10% starting weight with ~80% of mice surviving infection. Finally, CCxK18-hACE2 F1 progeny of three strains (CAST, NOD, WSB) had marked sexual dimorphism in response, with CAST and NOD F1 males susceptible to lethal disease, although differences in weight loss between sexes were not apparent. Yet another phenotype was obtained in SARS-CoV-2 infected WSB F1 progeny, where infection was uniformly lethal in females. Weight loss in WSB F1 males began later than other groups at 6 dpi and surviving males exhibited sustained weight loss until the end of the observation period in contrast to the rapid weight gain associated with recovery of other CCxK18-hACE2 F1 cohorts.

In K18-hACE2 mice, SARS-CoV-2 titers peak in the lung at 2-3 dpi and high titers of virus can be isolated from the CNS which may contribute to lethality in this model^{24,25}. Thus, virus replication kinetics were determined at 3 and 6 dpi in lung and brain of CCxK18-hACE2 F1 cohorts (Fig. 2A, 2B). F1 progeny of sensitive founder strains C57BL/6J and A/J showed high levels of infectious SARS-CoV-2 at 3dpi in lung homogenates that was reduced by 1-2 log₁₀ by 6 dpi with no differences between sexes. Infectious virus was generally not recovered from the CNS at 3dpi, but 50% of C57BL/6J and A/J F1 progeny had high virus burden in the CNS by 6dpi (10⁶-10⁸ PFU/g tissue). In contrast, peak lung virus titer in F1 progeny of the most resistant founder strain, PWK, was 150-fold lower than the sensitive strains at 3 dpi and was below the limit of detection at 6dpi. Control of virus replication in PWK F1s was also evident in the CNS where detection of infectious virus was sporadic. Interestingly, F1 progeny of additional CC

founder strains classed as resistant had equivalent peak viral titers in the lung to the sensitive K18-hACE2 and A/J F1 progeny, but they tended to control replication by 6 dpi to levels $1\log_{10}$ lower than sensitive mice and had less virus burden in the CNS. Finally, lung titers in (CAST, NOD and WSB)xK18-hACE2 F1 progeny were not different between males and females despite differences in clinical severity associated with sex. Thus, F1 progeny of CCxK18-hACE2 mouse strains can be further stratified into a) sensitive F1 progeny with high sustained virus replication in lung and CNS (C57BL/6J, A/J), b) resistant F1 progeny associated with lower peak virus titer and earlier control of replication in the lung with no or low dissemination to other organs (PWK, NZO, 129S1/J), and c) F1 progeny with sex bias where resistance is independent of virus titer in the lung suggesting a sex-based difference in host response (CAST, NOD, WSB). Relative expression of the K18-hACE2 transgene in lung suggested some variability, but expression was not associated with clinical phenotypes or peak viral burden (Fig. S2). Together, these data suggest that the range of clinical severity in mice is only partially associated with control of virus replication (Summarized in Table 1).

Pathological changes in SARS-CoV-2-infected K18-hACE2 mice were similar to those previously described^{7-9,26}. Inflammatory infiltrates evident by 3dpi included perivascular lymphocytes with alveolar septa thickened by neutrophils, macrophages and edema. At 6 dpi, pulmonary pathology was multifocal, and consistent with interstitial pneumonia including type II pneumocyte hyperplasia, septal, alveolar and perivascular inflammation comprised of lymphocytes, macrophages and neutrophils, with alveolar fibrin and edema evident. Bronchiolar pathology was not observed in these mice. Pathology was classified as none, mild (rare scattered inflammatory foci), moderate (coalescing inflammatory foci) or severe (widespread, large

inflammatory foci) as exemplified in Fig. 2c. The classification of ‘severe’ is a comparative term for these mice, as we generally did not observe pathology equivalent to that of the more severe Syrian hamster model of SARS-CoV-2 infection²⁷⁻²⁹. Surprisingly, in comparison to F1 progeny of sensitive founder strains (K18-hACE2 and A/JxK18-hACE2), F1 progeny of resistant PWK, female NZO and 129S/J mice, as well as those of NOD and WSB tended to have higher pathology scores in lungs (Fig. 2d). Lung distribution of viral RNA by RNAscope was limited to type I and II pneumocytes in all strains (Fig. 2b). Pathology in the CNS was scored as present or absent, as it generally consisted of subtle inflammatory foci, including perivascular cuffing and increased gliosis associated with necrotic cells observed in K18-hACE2 and DBA x K18-hACE2 F1 males (Fig. S3A-C, G). However, pathology in the CNS of CAST x K18-hACE2 F1 progeny was striking in that microthrombi were evident in capillaries with extensive hemorrhage in the absence of encephalitis, and was associated with viral RNA distribution in the same area (Fig. S3D-F).

In-depth longitudinal analyses of immune responses in patients with COVID-19 have defined key immunological correlates of disease outcome³⁰⁻⁴³. Moderate COVID-19 is associated with increased plasma levels of a core inflammatory signature of pro-inflammatory cytokines, chemokines and growth factors (e.g., IL-1 α , IL-1 β , IFN α , IL-12 p70, and IL-17A) that are effectively resolved⁴³. In contrast, severe COVID-19 includes sustained expression of these markers with additional signatures including IL-6, IL-10, IL-18, IL-23, TNF- α , and eotaxin among others suggesting that specific timing and failure to resolve inflammatory responses are important factors in disease progression⁴³. In SARS-CoV-2-infected CCxK18-hACE2 F1 mice, cytokines and chemokines were quantified in the serum and bronchoalveolar lavage fluid (BAL)

by multiplex analysis at 3 and 6 dpi. In general, cytokine levels were much higher in BAL fluid than in serum (Fig. S4A, B; individual data points are provided in Figs. S5-8). Strikingly in the BAL, comparison of sensitive (K18-hACE2 and A/JxK18-hACE2) versus resistant (PWK, NZO and 129S1)xK18-hACE2 F1 progeny revealed that high IFN α expression at 3dpi was associated with survival in both males and females (Fig. 3A, B). Resistant mice also expressed proinflammatory cytokine IL-6, Th1 cytokines (IL-12p70, IL-27) and chemokines (CXCL10, Gro- α /KC, CCL2, CCL3, CCL4, CCL5, CCL7 and CXCL2) (designated as Group A) in BAL at this early timepoint (Fig. 3B, Cluster 1). At 6 dpi, many Group A cytokines were resolving (Fig. 3C) while IFN- γ , TNF- α , IL-18, IL-1 β , IL-2, IL-4, IL-5, and IL-13 (designated as Group B) were added to the cytokine signature in resistant mice (Fig. 3B, Cluster 2). Interestingly, resistant PWK and NZO x K18-hACE2 mice exhibited a unique profile characterized by tempered levels of Group A and Group B along with high production of IFN γ , Gro- α /KC and eotaxin at 6dpi, a timepoint coincident with the most efficient clearance of virus from the lung (Cluster 4). In contrast, sensitive strains generally failed to produce Group A cytokines at 3 dpi (Cluster 3) and increases in production of Group A and Group B mediators in BAL occurred by 6 dpi (Fig. 3B, Cluster 2). Cytokine signatures were less clear in F1 progeny with sex bias (CAST, NOD and WSB) likely due to intermediate survival phenotypes observed in one or both sexes, although responses in BAL were generally higher in males (Fig. S4C). Together, these genetically diverse mice model major dynamic phenotypes observed in human COVID-19. Specifically, reduced disease severity in mice is associated with early type I IFN expression and a phased, controlled inflammatory response. In contrast, a delayed and unorchestrated innate response is associated with lethality.

Taken together, these observations demonstrate that use of host genetic variation in mice can model different outcomes of SARS-CoV-2 infection, addressing a major deficiency in the toolkit required to combat COVID-19. The phenotypes in diverse mice support a role for the innate immune system in determining COVID-19 severity^{32,44-47} and can be used to address key knowledge gaps, including mechanisms of innate immune control of virus replication, defining events needed for a well-orchestrated inflammatory response independent of early viral burden, molecular mechanisms of sex-dependent disease severity, and longer-term implications for tissue repair⁴⁸ and lung function.

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Declaration of Interests:

The authors have no interests to declare.

METHODS

Ethics statement

Animal study protocols were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at Rocky Mountain Laboratories (RML), NIAID, NIH in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the NIH. All animal experiments were performed in an animal biosafety level 3 (ABSL3) research facility at RML. Standard operating procedures for work

with infectious SARS-CoV-2 and protocols for virus inactivation were approved by the Institutional Biosafety Committee (IBC) and performed under BSL3 conditions.

Virus preparation

SARS-CoV-2 (USA_WA1/2020) from University of Texas Medical Branch (Vero passage 4) was propagated on Vero cells cultured in DMEM supplemented with 10% fetal bovine serum, 1 mM L-glutamine, 50U/ml penicillin and 50ug/ml streptomycin. Culture supernatants were collected at 72 hpi, aliquoted and stored at -80°C.

Mice and virus infection

CC founder x C57BL/6J -K18-hACE2 F1 were provided by The Jackson Laboratories and include the following: B6.Cg-Tg (K18-ACE2)2PrImn/J, 034860; (A/J x B6.Cg-Tg(K18-ACE2)2PrImn/J)F1/J, 035940; (PWK/PhJ x B6.Cg-Tg(K18-ACE2)2PrImn/J)F1/J, 035938; (NZO/HILtJ x B6.Cg-Tg(K18-ACE2)2PrImn/J)F1/J, 035936; (129S1/SvImJ x B6.Cg-Tg(K18-ACE2)2PrImn/J)F1/J, 035934; (CAST/EiJ x B6.Cg-Tg(K18-ACE2)2PrImn/J)F1/J, 035937; (NOD/ShiLtJ x B6.Cg-Tg(K18-ACE2)2PrImn/J)F1/J, 035935; (WSB/EiJ x B6.Cg-Tg(K18-ACE2)2PrImn/J)F1/J, 035939; (BALB/cJ x B6.Cg-Tg(K18-ACE2)2PrImn/J)F1/J, 035941; (BALB/cJ x B6.Cg-Tg(K18-ACE2)2PrImn/J)F1/J 035943. Six to 12 week-old male and female mice were inoculated by the intranasal route with 10^3 pfu of SARS-CoV-2 in a volume of 50ul PBS (Gibco). Prior to inoculation mice were anesthetized by inhalation of isoflurane. Mice were monitored daily for clinical signs of disease and weight loss.

Virus titration

Infectious virus in lung and brain tissue was quantified by plaque assay. Tissues were collected in 0.5ml of DMEM containing 2% FBS, 50U/ml penicillin and 50ug/ml streptomycin and immediately frozen.

Tissue samples were weighed, and then homogenized using 5mm steel beads and TissueLyzer II high-speed shaker (Qiagen). Ten-fold serial dilutions of homogenates were prepared in duplicate and used to inoculate Vero cells grown in 48-well tissue culture plates. Following 1 hour incubation at 37°C, the cells were overlaid with 1.5% carboxymethyl cellulose (CMC) in MEM and incubated at 37°C for 3-4 days. Cells were then fixed in 10% formalin and plaques were visualized by staining with 1% crystal violet diluted in 10% ethanol.

Measurement of SARS-CoV-2-specific IgG

Sera were collected at 21 dpi from mice that survived SARS-CoV-2 infection. SARS-CoV-2 spike protein-specific IgG was measured using SARS-CoV2 spike protein serological IgG ELISA kit (Cell Signaling Technology) per manufacturer's instructions.

Multiplex cytokine/chemokine analysis

Sera were collected from SARS-CoV-2-infected mice at 3 and 6 dpi by centrifugation of whole blood in GelZ serum separation tubes (Sarstedt). BAL samples were recovered by insufflation of lungs with 1 ml sterile PBS followed by aspiration to collect ~0.5ml volume of fluid. SARS-CoV-2 in sera and BAL fluid was inactivated by using γ -irradiation (2 MRad) and removed from the BSL3 laboratory. Cytokine concentrations in serum and BAL were measured using a 26-Procartaplex mouse cytokine/chemokine panel (ThermoFisher EPX260-26088-901) combined with a Simplex IFN α assay (ThermoFisher EPX01A-26027-901). Samples were run on a Luminex Bio-Plex 200 system with BioPlex Manager 6.1.1 software.

Histopathology and *in situ* hybridization

Tissues were fixed in 10% neutral buffered formalin for a minimum of 7 days with 2 changes according to IBC-approved standard operating procedure. Tissues were processed with a Sakura VIP-6 Tissue Tek,

on a 12-hour automated schedule, using a graded series of ethanol, xylene, and PureAffin. Embedded tissues were sectioned at 5 µm and dried overnight at 42°C prior to staining with hematoxylin and eosin. Chromogenic detection of SARS-CoV-2 viral RNA was performed using the RNAscope VS Universal AP assay (Advanced Cell Diagnostics Inc.) on the Ventana Discovery ULTRA stainer using a SARS-CoV-2 specific probe (Advanced Cell Diagnostics Inc. cat#848569). *In situ* hybridization was performed according to manufacturer's instructions.

Quantitative real-time PCR

Total lung RNA was extracted from homogenized lysates using the *mirVana* miRNA Isolation Kit (Invitrogen cat. AM1560) following the manufacturer's protocol for total RNA isolation. Total RNA samples were quantified using a Nanodrop spectrophotometer (Thermo cat. ND-2000C), and reverse transcribed to cDNA using the SuperScript IV VILO Master Mix with ezDNase enzyme (Invitrogen cat. 11766050) following the manufacturer's instructions. Expression of mouse *Ace2*, mouse *Gapdh*, and human ACE2 were quantified by qPCR using the ViiA7 Real-Time PCR System (Thermo cat. 44535545). TaqMan probes targeting mouse *Ace2* (Thermo probe ID Mm01159006_m1), mouse *Gapdh* (Thermo probe ID Mm99999915_g1), and human ACE2 (Thermo probe ID Hs01085333_m1) were used in conjunction with the TaqMan Fast Advanced Master Mix (Thermo cat. 4444556) and the ViiA7 Real-Time PCR System with QuantStudio Software (Thermo cat. 44535545). Samples were run in technical triplicate in 10ul reaction volumes on a 384-well plate, and m*Ace2* and hACE2 transcript abundance values were normalized to m*Gapdh* in each sample and relative expression was calculated using a common reference lung sample (C57BL/6J male) and the delta delta-Ct method.

Statistical analysis

Comparison of survival curves in males versus females for each strain was performed using the log-rank (Mantel-Cox) test. One-way analysis of variance (ANOVA) with Tukey's multiple comparison posttest was used to compare cytokine and chemokine levels of BAL and serum. Differences between groups were

considered significant at a P value of <0.05 . All statistical analyses were performed with graphPad Prism 8.0 (GraphPad Software) or Qlucore Omics Explorer Version 3.7 (Qlucore AB).

Figure Legends:

Figure 1: CCxK18-hACE2 F1 progeny mice demonstrate a range of clinical severity following infection with SARS-CoV-2. Mice were infected intranasally with 10^3 PFU of SARS-CoV-2 and monitored for survival and weight loss. F1 founder strains were classified as: **a**, sensitive with 50% or greater mortality and no difference between males and females, **b**, resistant with 35% or less mortality, or **c**, sensitive with a sex bias as having statistically significant difference between sensitivity of male and female mice. Mice without evidence of seroconversion at the end of the study were removed from analysis (N=6-8/per sex).

Figure 2: Virus replication kinetics and pathology in CCxK18-hACE2 F1 progeny. **a**, Virus titers (PFU/g tissue) in lung and brain of male and female mice were measured at 3 and 6 dpi. **b**, RNAscope of SARS-CoV-2 genome predominantly localizing to pneumocytes in areas of lung inflammation. Serial sections demonstrate pathology (upper) and viral RNA distribution (lower; Red). Scale bars represent 100 μ m. **c**, Examples of pathology scoring used in this study. Scale bars represent 500 μ m. **d**, Lung pathology scores for CC Founder F1 males and females at 3 and 6 dpi. N = 6/time point/sex. ND, not determined.

Figure 3: Timing of type I IFN and inflammatory cytokine responses in the lung is associated with clinical severity in CCxK18-hACE2 F1 progeny. **a**, IFN α protein in BAL

from resistant and sensitive mice. **b**, cytokine and chemokine responses in BAL at 3 and 6 dpi. **c**, Resolving group A cytokines in mice resistant to lethality, but not sensitive mice.

Supplemental Figure 1: Survival, weight loss and SARS-CoV-2 replication kinetics in BALB/cxK18-hACE2 or DBA x K18-hACE2 F1 progeny. **a**, Survival and weight loss in BALB/cxK18-hACE2 F1. **b**, Survival and weight loss in DBA x K18-hACE2 F1. **c**, Virus titers (PFU/g tissue) in lung and brain of male and female mice were measured at 3 and 6 dpi. **d**, Cytokine expression in BAL.

Supplemental Figure 2: Relative expression of human *ACE2* in CCxK18-hACE2 F1 progeny. Gene expression was measured by real-time RT-PCR and expressed relative to that in K18-hACE2 mice.

Supplemental Figure 3: Examples of pathology in the brain of SARS-CoV-2-infected CCxK18-hACE2 F1 progeny. **a**, Small focal areas of inflammation were most commonly observed (circled). Scale bar = 1000µm. **b**, increased magnification of **a**. demonstrating perivascular cuffing, gliosis, and evidence of necrotic cells in areas of focal inflammation. Scale bar = 200µm. **c**, Example of a pronounced lymphocytic inflammatory response with perivascular cuffing and increased cellularity (arrowheads). Scale bar = 100µm. **d**, Microthrombus occluding cerebral capillaries and hemorrhage (arrow) observed in CASTxK18-hACE2. Scale bar = 100µm. **e**, Example of extensive hemorrhage in the brain of CASTxK18-hACE2 F1 progeny

associated with extensive virus replication as demonstrated in f. Scale bar = 1000µm. **f**, serial section relative to e. showing extensive SARS-CoV-2 genome by RNAscope. Scale bar = 1000µm. **g**, Incidence of encephalitis for CC Founder F1 males and females at 3 and 6 dpi. ND, not determined.

Supplemental Figure 4: Interferon, cytokine and chemokine expression in the serum compared to the BAL of SARS-CoV-2-infected female CCxK18-hACE2 F1 progeny.

Cytokines were measured at 3 and 6 dpi by bioplex. **a**, 3 dpi, and **b**, 6dpi for sensitive and resistant strains with no sex-bias. **c**, 3 and 6 dpi from strains with a sex-bias.

Supplemental Figure 5: Interferon, cytokine and chemokine expression in the bronchoalveolar lavage from SARS-CoV-2-infected male CCxK18-hACE2 F1 progeny.

Cytokines were measured at 3 and 6 dpi by bioplex. The average cytokine expression in BAL of naïve mice is indicated by a grey dashed line.

Supplemental Figure 6: Interferon, cytokine and chemokine expression in the serum of SARS-CoV-2-infected male CCxK18-hACE2 F1 progeny. Cytokines were measured at 3 and 6 dpi by bioplex. The average cytokine expression in serum of naïve mice is indicated by a grey dashed line.

Supplemental Figure 7: Interferon, cytokine and chemokine expression in the bronchoalveolar lavage from SARS-CoV-2-infected female CCxK18-hACE2 F1 progeny.

Cytokines were measured at 3 and 6 dpi by bioplex. The average cytokine expression in BAL of naïve mice is indicated by a grey dashed line.

Supplemental Figure 8: Interferon, cytokine and chemokine expression in the serum of SARS-CoV-2-infected female CCxK18-hACE2 F1 progeny. Cytokines were measured at 3 and 6 dpi by bioplex. The average cytokine expression in serum of naïve mice is indicated by a grey dashed line.

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464 **Table 1:** Summary of percent survival, weight loss, virus burden and BAL cytokine profiles in
 465 K18-hACE2 x CC Founder F1 progeny.

Strain	Sex	%Survival	Mean time to death (days)	Peak weight loss		Average Virus Titers in Lung		IFN α in BAL	Cytokine profile BAL	
				%	DPI	3 dpi Mean (Std Dev)	6 dpi Mean (Std Dev)		3 dpi	6 dpi
K18-hACE2	M	14	6	16.7	7	1.5e6 (1.9e6)	7.6e4 (1.3e5)	Low	No/Low	High Group A and Group B
	F	29	7	12.0	7	1.5e6 (1.4e6)	8.2e3 (5.7e3)	Low		
A/J x K18-hACE2 F1	M	33	8	15.0	7	2.8e6 (2.2e6)	4.65e4 (6.9e4)	Low	No/Low	High Group A and Group B
	F	66	UD	6.1	7	3.3e6 (2.4e6)	3.0e4 (2.9e4)	Low		
PWK x K18-hACE2 F1	M	83	UD	-	-	1.6e5 (2.3e5)	BLD	High	High Group A	Moderate Group A and Group B
	F	83	UD	7.0	7	1.5e4 (2.3e4)	BLD	Low		
NZO x K18-hACE2 F1	M	86	UD	13.9	9	2.6e6 (2.9e6)	3.8e3 (3.7e3)	High	High Group A	High Group A and B
	F	71	UD	21.9	9	2.0e6 (9.4e5)	1.3e3 (1.0e3)	High		
129S1J x K18-ACE2 F1	M	86	UD	11.8	7	1.1e6 (8.3e5)	3.0e3 (2.8e3)	High	High Group A	High Group A and Group B
	F	71	UD	12.0	7	8.1e5 (6.4e4)	2.4e4 (4.0e4)	Moderate		
CAST x K18- ACE2 F1	M	0	6	17.2	7	3.4e6 (3.2e6)	4.8e5(3.7e5)	Low	Low Group A	Low Group A Males = Females
	F	50	15.5	35.8	8	1.75e6(1.5e6)	3.4e4 (2.9e4)	Low		
NOD x K18-ACE2 F1	M	33	6.5	6.0	6	1.7e6 (3.7e6)	4.8e3 (5.3e3)	Low	No/Low	Group A and Group B Males > Females
	F	83	UD	-	-	4.3e5 (7.4e5)	6.4e3 (8.1e3)	Low		
WSB x K18-ACE2 F1	M	66	UD	32.0	11	4.2e6 (1.8e6)	3.1e4 (1.6e4)	High	Moderate Group A	Group A and B Males > Females
	F	0	8	25.1	8	3.7e6 (4.1e6)	2.5e4 (1.2e4)	High		
BALB/c x K18-ACE2 F1	M	100	UD	3.0	5	6.0e5 (5.5e5)	1.6e4 (1.3e4)	Moderate	No/Low	Group A and B
	F	66	UD	8.3	6	4.4e5 (4.3e5)	6.0e2 (1.2e3)	High	High Group A	Females > Males
DBA/2 x K18-ACE2 F1	M	57	UD	16.0	10	1.5e6 (1.7e6)	1.7e4 (2.2e4)	Moderate	No/Low	Group A and B Females > Males
	F	71	UD	3.6	6	2.0e6 (1.0e6)	1.2e3 (1.0e3)	Moderate		

466 UD: undetermined

467 BLD: below limit of detection

Fig. 1

Figure 2

Fig. S1

Fig. S2

Male BAL cytokine panel

Fig. S5

Male serum cytokine panel

Fig. S6

Female BAL cytokine panel

Fig. S7

Female serum cytokine panel

Fig. S8