**Supplementary Data**

**IL-1 Mediates Tissue Specific Inflammation and Severe Respiratory Failure In Covid-19: Clinical And Experimental Evidence**

Running heading: IL-1 bioactivity in COVID-19

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**Figure S1 Lung pathology of the studied experimental COVID-19 pneumonia model**

Mice were sacrificed and lung sections were stained with heamtoxylin-eosin. At least 10 fields were scored from 0 to 2 each field for: A) the degree of presence of neutrophils in the alveoli; B) the degree of the presence of alveoli in the interstitial space; and C) the degree of thickening of the of the alveolar septa. Panels A to C indicate the mean of the total degree of pathology of each animal. Characteristic lung histology sections are shown: D) x10 of one animal challenged with plasma from COVID-19 patient showing diffuse neutrophil and mononuclear infiltration and capillary congestion; E) x25 of one other animal challenged with plasma from another COVID-19 patient showing more dense infiltration and vascular congestion; and F) x10 of one animal challenged with plasma from one healthy individual

Only statistically significant differences by the Mann Whitney U test are shown: \*p<0.05; \*\*p<0.01

Chart, box and whisker chart

Description automatically generated**Figure S2 Dexamethasone attenuates the effect of IL-1a inhibition on the compartmentalized hyperinflammation in a COVID-like murine model**

In a COVID- like infection model, C57Bl6 mice were challenged intravenously (i.v.) with plasma of healthy volunteers (HV) or patients with ARDS due to COVID-19 for three consecutive days. In separate experiments, on each day of plasma challenge, mice were treated with Anakinra; dexamethasone or their combination. Mice were sacrificed on day 4.A-C) Tumor necrosis factor alpha (TNFα); D-E) Interleukin (IL) -6; F) Interferon gamma (IFNγ) and G-I) myeloperoxidase (MPO) activity was determined in tissues. Comparison by the Mann Whitney U test; ns non-significant; ✱ p< 0.05; ✱✱ p< 0.01.

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**Figure S3 IL-1 inhibition does not affect Th17 responses in COVID-19**

A-B) In a COVID- like infection model, C57Bl6 mice were treated intravenously (i.v.) with plasma of healthy volunteers or patients with ARDS due to COVID-19 for three consecutive days.

C-D) In a COVID- like infection model, C57Bl6 mice were treated intravenously (i.v.) with plasma of patients with ARDS due to COVID-19 with or without treatment with the IL-1 receptor inhibitor anakinra for three consecutive days.

Mice were sacrificed on day 4.Splenocytes were isolated and incubated with *C. albicans*. Th17 response is shown. Comparison by the Mann Whitney U test; ns non-significant. Treatment of mice with anakinra did not change the ex vivo production capacity of IL- 17A or IL- 22 indicating a lack of correlation with Th17 responses.



**Figure S4 SARS-CoV spike protein is a weak stimulus of cytokine production in a COVID-like murine model**

A-B) In a COVID- like infection model, C57Bl6 mice were treated intravenously (i.v.) with plasma of healthy volunteers or patients with ARDS due to COVID-19 with or without treatment with the IL-1 receptor inhibitor anakinra for three consecutive days.

Mice were sacrificed on day 4.Splenocytes were isolated and incubated with SARS-CoV-2 spike protein for the production of interferon-gamma (IFNγ), tumour necrosis factor-alpha (TNFα) and interleukin (IL)-6. Comparison by the Mann Whitney U test; ns non-significant.

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**Figure S5 Pathways associated with the studied animal model of COVID-19**

In a COVID- like infection model, C57Bl6 mice were treated intravenously (i.v.) with plasma of healthy volunteers or patients with ARDS due to COVID-19. After three days of treatment, mice were sacrificed and transcriptomic analysis was done in sections of the lung and spleen. Bioanalysis denoted four up-regulated genes in the lung and one down-regulated gene in the spleen after injection with plasma of COVID patients. A Table with the implicated pathways is also provided.



**Figure S6 Lack of correlation between cytokine production in tissues and number of viral transcripts**

The number of transcripts of the *ORF1 ab* gene and *N* gene of SARS-COV-2 was determined in plasma of patients with ARDS due to COVID-19. In a COVID- like infection model, C57Bl6 mice were treated intravenously (i.v.) with the same plasma from patients with ARDS due to COVID-19 for three consecutive days. Correlation of number of transcripts of the ORF1 ab gene (Α – D) and n gene (E – H) with TNFα, IL- 6, IFNγ and MPO in the lung. Spearman rank correlation coefficients (rs), interpolation lines and p- values are given.

Chart, box and whisker chart

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**Figure S7 COVID- 19 is associated with increased susceptibility to secondary infection due to *A. baumannii***

Percentage of critically ill COVID- 19 patients who developed secondary bacteremia by Gram- negative bacteria within 28 days after admission, sorted by type of bacteria. Comparisons by the Fisher exact test. ✱ p< 0.05; ✱✱ p< 0.01; ✱✱✱✱ p< 0.0001.

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| **Table S1. Baseline clinical and laboratory characteristics of critically ill patients with pneumonia by SARS-CoV-2 coronavirus according to development of secondary bacteremia or not** | | | | |
|  | *A. baumannii* bacteremia  (n=11) | Other gram-negative  bacteriemia  (n=3) | No secondary bacteremia  (n=120) | p-value |
| Age (years, mean ± SD) # | 66.11 ± 9.75 | 63.00 ± 1.73 | 63.92 ± 13.57 | 0.884 |
| CCI (mean ± SD) # | 3.00 ± 1.80 | 2.33 ± 0.58 | 2.79 ± 1.81 | 0.854 |
| APACHE II score (mean ± SD) # | 11.89 ± 5.01 | 9.33 ± 5.25 | 10.26 ± 5.18 | 0.663 |
| SOFA (mean ± SD) # | 5.67 ± 1.87 | 6.00 ± 2.65 | 6.10 ± 2.88 | 0.907 |
| Laboratory values (mean ± SD) | | | | |
| White blood cells (mm3) | 13620 ± 5085 | 11777 ± 3487 | 10095 ± 5252 | 0.206# |
| Absolute neutrophil counts (mm3) | 11950 ± 4794 | 10695 ± 3678 | 8522 ± 4956 | 0.167# |
| Absolute lymphocyte counts (mm3) | 951 ± 739 | 689 ± 219 | 913 ± 629 | 0.819# |
| Platelets (x 103, mm3) | 289428 ± 65739 | 263000 ± 32741 | 253731 ± 108144 | 0.683# |
| C-reactive protein (mg/l) | 154.72 ± 123.90 | 142.77 ± 105.96 | 137.54 ± 215.62 | 0.984# |
| Ferritin (ng/ml) | 1502 ± 1844 | 815 ± 566 | 1439 ± 1786 | 0.829# |
| Procalcitonin (ng/ml) | 042 ± 0.65 | 0.13 ± 0.05 | 0.98 ± 1.96 | 0.694# |
| D-dimers (ng/ml) | 18571 ± 38008 | 1046 ± 631 | 3715 ± 16917 | 0.222# |
| AST (U/l) | 49.21 ± 63.40 | 48.00 ± 34.66 | 51.51 ± 41.31 | 0.982# |
| ALT (U/l) | 35.80 ± 23.75 | 44.33 ± 24.38 | 52.68 ± 57.96 | 0.727# |
| Creatinine (mg/dl) | 0.81 ± 0.33 | 0.77 ± 0.06 | 1.26 ± 1.60 | 0.667# |
| Abbreviations: SD: standard deviation; APACHE: acute physiology and chronic health evaluation; CCI: Charlson’s comorbidity index; SOFA: sequential organ failure; AST: aspartate aminotransferase; ALT: alanine aminotransferase.  Comparisons by one-way ANOVA with the Bonferroni correction. | | | | |

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