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Nan Xiao

Tsinghua University

Meng Nie

Tsinghua University

Huanhuan Pang

Tsinghua University

Bohong Wang

Tsinghua University

Jieli Hu

Chongqing Medical University

Xiangjun Meng

Tsinghua University

Ke Li

Chinese Academy of Medical Sciences & Peking Union Medical College

Xiaorong Ran

Agilent Technologies (China),

Quanxin Long

Chongqing Medical University

Haijun Deng

Chongqing Medical University

Ni Tang (✉ nitang@cqmu.edu.cn)

Chongqing Medical University

Ailong Huang (✉ ahuang@cqmu.edu.cn)

Chongqing Medical University

Zeping Hu (✉ zeping_hu@tsinghua.edu.cn)

Tsinghua University

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Host metabolite-cytokine correlation landscape in SARS-CoV-2 infection

Nan Xiao^{1,7}, Meng Nie^{1,2,7}, Huanhuan Pang^{1,2,7}, Bohong Wang^{1,7}, Jieli Hu^{3,7}, Xiangjun Meng¹, Ke Li⁴, Xiaorong Ran⁵, Quanxin Long³, Haijun Deng³, Ni Tang^{3*}, Ailong Huang^{3*}, and Zeping Hu^{1,2,6,8*}

¹School of Pharmaceutical Sciences, Tsinghua University, Beijing, 100084, China.

²Tsinghua-Peking Joint Center for Life Sciences, Tsinghua University, Beijing, 100084, China.

³Key Laboratory of Molecular Biology for Infectious Diseases (Ministry of Education), Chongqing Medical University, Chongqing, China.

⁴NHC Key Laboratory of Biotechnology of Antibiotics, Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, 100050, China

⁵Agilent Technologies (China), No. 3, Wang Jing Bei Lu, Chaoyang District, Beijing, 100102, China

⁶Beijing Frontier Research Center for Biological Structure, Tsinghua University, Beijing, 100084, China.

⁷These authors contributed equally

⁸Lead Contact

*Correspondence:

nitang@cqmu.edu.cn (N.T.), ahuang@cqmu.edu.cn (A.H.), zeping_hu@tsinghua.edu.cn (Z.H.)

Abstract

The systemic cytokine release syndrome (CRS) is a major cause of the multi-organ injury and fatal outcome induced by SARS-CoV-2 infection in severe COVID-19 patients. It has been well-known that metabolism plays a role in modulating the immune responses in infectious diseases. Yet, how the host metabolism correlates with CRS in COVID-19 patients and how the perturbed metabolites affect the cytokine release remains unclear. Here, we performed both metabolomics and cytokine/chemokine profiling on serum samples from the same cohort of healthy controls, mild and severe COVID-19 patients and delineated the global metabolic and immune response landscape along disease progression. Intriguingly, the correlation analysis revealed the tight link between metabolites and proinflammatory cytokines and chemokines, such as IL-6, M-CSF, IL-1 α , IL-1 β , implying the potential regulatory role of arginine metabolism, tryptophan metabolism, and purine metabolism in hyperinflammation. Importantly, we demonstrated that targeting metabolism markedly modulated the proinflammatory cytokines release by PBMCs isolated from SARS-CoV-2-infected rhesus macaques *ex vivo*. Beyond providing a comprehensive resource of metabolism and immunology data of SARS-CoV-2 infection, our study showed that metabolic alterations can be potentially exploited to develop novel strategy for the treatment of fatal CRS in COVID-19.

Introduction

Coronavirus disease 2019 (COVID-19), caused by highly infectious severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has recently become global pandemic¹, highlighting an urgent need for effective therapeutic strategies. SARS-CoV-2 infection triggers immune response contributing to both virus clearance and acute respiratory distress syndrome (ARDS) development^{2,3}. The severe COVID-19 patients often experience cytokine release syndrome (CRS) referred as “cytokine storm”, which is characterized by excessive proinflammatory cytokines production and leads to widespread damage, multiple organ failure, and fatal clinical outcomes⁴⁻⁸. Emerging clinical trials uncover that immunomodulatory drugs, such as the IL-6 receptor-blocking antibody tocilizumab and JAK1/2 inhibitor ruxolitinib⁹⁻¹¹, can dampen the hyperactive immune response, suggesting cytokine-release blockade as a promising treatment option. Hence, identifying the key factors driving CRS induced by SARS-CoV-2 infection is of utmost importance to provide fresh insights for novel immunomodulatory therapies.

The association of metabolism and immunity has been reported since 1960s¹², and many studies have shown that metabolic pathways can regulate the innate and adaptive host responses to the infection of various viruses, such as human immunodeficiency virus (HIV)¹³, yellow fever virus¹⁴, severe fever with thrombocytopenia syndrome virus (SFTSV)¹⁵⁻¹⁷. Since the COVID-19 pandemic, studies exploring the immunological and metabolic signatures in patients have been reported extensively^{5,7,18,19}. However, the host

metabolism-immune response correlation and the potential modulatory role of the perturbed metabolism in immune response upon SARS-CoV-2 infection remains largely unknown. Here we performed both metabolomics and cytokine/chemokine profiling in serum samples from the same cohort of COVID-19 patients and healthy controls. Strikingly, correlation analysis of metabolites and cytokines revealed the potential metabolic determinants of hyperinflammation to SARS-CoV-2 infection. Importantly, targeting arginine metabolism, tryptophan metabolism, and purine metabolism by metabolite supplementation or pharmacological inhibition modulated the *ex vivo* inflammatory cytokine secretion by isolated peripheral blood mononuclear cells (PBMCs) derived from SARS-CoV-2-infected rhesus macaques, indicating that metabolic interventions may be potentially exploited as rational strategies to suppress CRS induced by COVID-19.

Results

Study design and patient cohort

To understand how the host metabolism correlates with CRS in COVID-19 patients and how the perturbed metabolites may affect the cytokine release, we performed both metabolomics and cytokine profiling on serum samples from the same COVID-19 patient cohort. The cohort comprises of 17 healthy controls, 14 mild and 23 severe COVID-19 patients, as well as 7 mild patients with longitudinal follow-up time-points that occurred 4-36 days post symptom onset. We collected the clinical information and conducted immunological and biochemical laboratory test as well as metabolomics and cytokine profiling on the serum samples from these patients. To ascertain the potential modulatory role of metabolism in cytokine secretion, we performed correlation analysis between metabolites and cytokines, and validated the functional effect of metabolic intervention on cytokine secretion *ex vivo* by isolated peripheral blood mononuclear cells (PBMCs) derived from SARS-CoV2-infected rhesus macaque (**Fig. 1a**). The basic clinical features of the cohort are detailed in **Supplementary Table S1**. Significant reductions in lymphocyte count, and marked increase in C-reactive protein (CRP), alanine amino-transferase (ALT), aspartate aminotransferase (AST), direct bilirubin (DBIL) and glucose were exhibited in severe COVID-19 patients, which are consistent with previous findings^{1,20} (**Extended Data Fig. 1**).

Metabolic profiling of serum samples from COVID-19 patients

To determine the metabolic perturbations associated with SARS-COV-2 infection, we profiled the serum samples from 17 healthy controls and 44 COVID-19 patients by using both targeted and untargeted metabolomics analyses (**Extended Data Fig. 2a**). Targeted metabolomics analysis was performed on an ultra-high-performance liquid chromatography-triple quadrupole mass spectrometry system (UHPLC-MS/MS). A total of 258 metabolites were monitored and 145 metabolites were reliably detected. Untargeted metabolomics analysis was performed on an UHPLC quadrupole TOF high resolution MS/MS system. In total 6072 metabolite features were extracted from the raw data acquired with positive and negative ionization modes and 1910 features were consistently detected. Among these features, 161 metabolites were identified based on their accurate mass, retention time, isotopic patterns and MS/MS spectra. By integrating the targeted and untargeted metabolomics datasets, we identified in total 253 metabolites and observed distinct metabolic profiles among healthy controls, mild and severe patients (**Fig. 1b**, **Extended Data Fig. 2b-d**). Volcano plots highlighted 109 differential metabolites between healthy controls and mild patients, and 98 differential metabolites between healthy controls and severe patients, reflecting markedly dysregulated metabolic state of COVID-19 (**Fig. 1c, d**).

To characterize the dysregulated metabolic pathways in COVID-19 patients compared to healthy controls, we performed pathway enrichment analyses and observed that primary

bile acid biosynthesis, taurine and hypotaurine metabolism, amino acid metabolism, nucleic acid metabolism, and citrate cycle (TCA cycle) were significantly perturbed in both mild and severe patients (**Fig. 1e**). In contrast, metabolic pathways such as branched-chain amino acid biosynthesis, glutathione metabolism, and pyruvate metabolism were altered merely in mild patients, whereas thiamine metabolism, glutamine and glutamate metabolism, and nitrogen metabolism were significantly changed specifically in severe patients (**Fig. 1e**). Interestingly, the metabolites that displayed constant upward or downward trend along disease progression were mostly associated with purine metabolism, nicotinate and nicotinamide metabolism, tryptophan metabolism, TCA cycle and arginine metabolism (**Extended Data Fig. 2e**). Intermediates in arginine metabolism have been regarded as regulators of lymphocyte-suppression during immune response ²¹. Our previous work has demonstrated that arginine deficiency was associated with T cell dysregulation in severe fever with thrombocytopenia syndrome virus (SFTSV) infection ¹⁵. Here we observed that glutamate and aspartic acid were upregulated, while glutamine and citrulline were downregulated along disease progression (**Extended Data Fig. 2f**). Succinate, an intermediate of TCA cycle that has been proved to be an innate immune signaling molecule during inflammation in macrophage ²², displayed a successive increase along disease severity (**Extended Data Fig. 2g**). Moreover, the increase in kynurenine and decline in tryptophan and serotonin suggested the enhanced activity of rate-limiting enzyme indole 2,3-dioxygenase (IDO1) (**Extended Data Fig. 2h**), which was reported to be a modulator of inflammation ^{23,24}. In addition, NAD⁺ metabolism was found to be altered

by host-pathogen interactions during innate and adaptive immune responses²⁵, including SARS-COV-2 infection²⁶. Here the level of nicotinamide mononucleotide (NMN), a key metabolite in the NAD⁺ metabolism, decreased as the severity of COVID-19 increases (**Extended Data Fig. 2i**). Taken together, our data delineated the global metabolic alterations along the increase in the severity of COVID-19.

Cytokines secretion correlates with altered metabolism in COVID-19 patients

Extensive studies proved that metabolic pathways can control the innate and adaptive immune response during viral infection^{13,27}. To clarify the modulatory role of metabolism in inflammation after SARS-CoV-2 infection, we assessed the cytokine and chemokine levels in COVID-19 patients and healthy controls. Consistent with previous studies reported^{5-8,28}, COVID-19 patients presented marked elevation for most cytokines and chemokines compared to healthy controls (**Extended Data Fig. 3a**). Particularly, the CRS-related cytokines, including IL-6, IL-1 β , IL-10, IL-18 and IFN- γ , displayed progressive increase along disease progression from healthy controls to mild and severe patients (**Extended Data Fig. 3b**), highlighting the broad and strong inflammatory response in severe COVID-19 patients.

In order to identify the metabolic alteration that might contribute to cytokine secretion in serum of COVID-19 patients, we analyzed the correlation between the cytokines and metabolites using linear regression after adjusting for age and gender. Systematic pathway

analysis revealed a surprising number of significant correlations of cytokine alterations with dysregulated metabolic pathways, mainly including amino acid metabolism, nucleic acid metabolism, and energy metabolism (**Fig. 2a**). Moreover, most inflammatory cytokines strongly correlated with metabolites involved in arginine metabolism, tryptophan and NAD⁺ metabolism, purine and pyrimidine metabolism, cysteine and methionine metabolism, TCA cycle and primary bile acid metabolism (**Fig. 2b-e**). These observations provide evidence that host metabolism broadly and highly correlated with cytokines linked to CRS. Notably, the metabolites involved in arginine metabolism (i.e., arginine, glutamine, guanidineacetic acid, creatinine, aspartic acid) displayed strongly negative correlation with CRS-related cytokines (i.e., IL-6, M-CSF, IL-18, MCP-1, IL-12 p70, IFN- α 2) (**Fig. 2f**). In addition, purine metabolism (i.e., xanthine, guanosine, adenine) exhibited negative correlation with CRS-related cytokines (i.e., IL-6, MCP-3, GM-CSF, IL-1 α , IL-1 β) (**Fig. 2g**). Metabolites involved in tryptophan and NAD⁺ metabolism, such as kynurenine and NMN, showed positive correlation with MCP-3, M-CSF and IL-6. In contrast, tryptophan and serotonin had negative correlation with IFN- γ and MCP-1 (**Fig. 2h**). The correlations between metabolites and cytokines in mild and severe patients suggest that the disturbances in these metabolic pathways might potentially contribute to the hyperinflammation in COVID-19.

Longitudinal metabolite-cytokine correlation in follow-up mild COVID-19 patients

To further identify the dynamic correlations between metabolites and cytokines at longitudinal stages after SARS-CoV-2 infection, we performed c-means clustering analysis on both metabolite and cytokine data from the hospitalized mild patient with 4-36 follow-up time-points. We identified four main clusters of longitudinal trajectories which characterized distinct metabolic and immune signatures in these patients with acute antibody responses to SARS-CoV-2 infection ²⁹ (**Extended Data Fig. 4a, b**). Molecules enriched in cluster 1 increased at symptoms onset but gradually decreased during hospitalization; molecules in cluster 2 exhibited a sharp decreased at symptoms onset and sustained stable levels in later time-points. However, molecules in cluster 3 sustained steady levels but presented a delayed elevation in the very late events; cluster 4 contained molecules that elevated gradually and declined in late phases (**Fig. 3a**).

Three CRS-related cytokines including IL-6, IP-10 and M-CSF belonged to cluster 1 (**Fig. 3b**). Notably, the levels of IL-6, which is highly correlative to CRS ³⁰, decreased during the first two weeks of symptom onset and remained at low level in later phases (**Fig. 3b, Extended Data Fig. 4c**). The IFN- γ inducible protein, IP-10/CXCL-10, is considered as a member of CXC chemokine family with proinflammatory and severity-related properties in COVID-19 ⁸. The levels of IP-10 showed a sharp decline in the initial phase of treatment and sustained at a relatively low levels during hospitalization (**Fig. 3b, Extended Data Fig. 4c**). Consistently, the myeloid cytokine M-CSF also showed a downward trend over the course of mild disease similar to IP-10 (**Fig. 3b, Extended Data Fig. 4c**). In addition,

proinflammatory cytokines in cluster 2, including G-CSF, IL-8, MIP-1 α and MCP-3, also showed decreased levels in mild patients compared to healthy controls and remained steady levels during hospitalization (**Fig. 3b, Extended Data Fig. 4c**). However, proinflammatory cytokines in cluster 3 (i.e., IL-17A and TNF- β) and cluster 4 (i.e., IL-1 α , IL-1 β , IL-18 and MCP-1) showed the upward trend along the follow-up time-points (**Fig. 3b, Extended Data Fig. 4c**). These observations indicate that alleviation of inflammatory immune responses may be accompanied with clinical recovery in hospitalized mild patients.

Interestingly, several cytokines enriched in cluster 4 were associated with suppression of the inflammatory responses and viral replication, such as IL-10 and IFN- α 2 (**Fig. 3b, Extended Data Fig. 4c**). IL-10, which reportedly plays a role in antagonizing inflammatory cell populations and suppressing immune hyperactivity³¹, continued to elevate over time and maintained at high levels (**Fig. 3b, Extended Data Fig. 4c**). Also, IFN- α 2 is reported to play a crucial role in combating infection through inhibiting viral replication and preventing viral entry into neighboring cells, thus used for treating several viral infections, including hepatitis B and C³². We observed the steadily elevated and sustained levels of IFN- α 2 over hospitalization (**Fig. 3b, Extended Data Fig. 4c**). The increased levels of IL-10 and IFN- α 2 may reflect the presence of a negative feedback loop to control the inflammatory response and virus infection. These data suggest that a protective immune response may occur along with downward trend of proinflammatory cytokines and clinical recovery in hospitalized mild patients³³.

We next characterized metabolites that were enriched in four clusters and specific metabolite-cytokine correlations. Interestingly, metabolites associated with arginine metabolism were enriched in cluster 2 and cluster 3 (**Fig. 3c**). We observed the upward trend of arginine, ornithine, glutamate and proline, whereas the decrease in citrulline (**Fig. 3d; Extended Data Fig. 5a, b**). Arginine metabolism reportedly played a crucial role in regulation of immune responses^{15,21}. Correlation analysis showed that intermediates in arginine metabolism highly correlated with proinflammatory cytokines including IL-6, M-CSF and MIP-1 β (**Fig. 3e**). Dysregulated tryptophan metabolism and NAD⁺ metabolism was evident by a marked alteration in associated metabolites exhibited four trajectories (**Fig. 3c**). The increased metabolites (i.e., tryptophan, indole) and decreased metabolites (i.e., kynurenine, kynurenic acid) reflected a hyperactivation of tryptophan-kynurenine pathway (**Fig. 3d, Extended data Fig. 5a, b**), which plays an important role in modulating inflammation²³. Moreover, kynurenine positively correlated with proinflammatory cytokines including IP-10, MCP-3 and M-CSF, whereas tryptophan negatively correlated with MIP-1 α , suggesting the regulatory role of tryptophan metabolism in inflammatory response (**Fig. 3e**). Additionally, a large proportion of intermediates in purine metabolism displayed the upward trend such as inosine and adenine (**Fig. 3d, Extended data Fig. 5a, b**), and showed the negative correlation with most proinflammation cytokines (**Fig. 3e**). Taken together, our data identify a panel of metabolite-cytokine correlation, which may provide an unbiased way to determine the potential metabolic regulators of proinflammatory cytokine secretion.

Effects of modulating metabolism on cytokine release by PBMCs *ex vivo*

Our data presented above delineated the strong correlation between cytokines and metabolites, and identified metabolic pathways that are potentially crucial for proinflammatory cytokine production. We next evaluated whether intervening arginine metabolism, tryptophan metabolism and purine metabolism could regulate cytokine induction. To this end, we isolated PBMCs from SARS-CoV-2-infected and mock-infected rhesus macaques, and measured the cytokine concentrations after treatment with metabolites or compounds interfering these key metabolic pathways (**Fig. 4a**). Interestingly, we observed that supplementation of arginine markedly inhibited the SARS-CoV-2-induced proinflammatory cytokines production by PBMCs, most of which linked to CRS including IL-1 α , IL-1 β , IL-2, IL-6, TNF- α , GM-CSF, G-CSF and MIP-1 α (**Fig. 4b**, **Extended Data Fig. 6**). Notably, the elevated level of IL-10, which is responsible for inhibition of proinflammation cytokines production from macrophage and dendritic cell (DC) populations³¹, was also suppressed (**Fig. 4b**). These observations suggest that serum arginine metabolism may play a modulatory role in the hyperinflammation, thus could be exploited as a potential therapeutic target for CRS in COVID-19.

The conversion of tryptophan into kynurenine in immune cells is finely regulated by the enzyme indoleamine 2,3 dioxygenase 1 (IDO1), which is reportedly involved in regulating hyperinflammatory responses²⁴. The elevated ratio between circulating kynurenine and tryptophan (Kyn/Trp) in patient's serum described above suggested an increased activity

of IDO1. Addition of epacadostat, an IDO1 inhibitor, suppressed the SARS-CoV-2-induced proinflammation cytokine release including IL-1 α , IL-1 β , IL-6, TNF- α , GM-CSF, G-CSF, IL-17A and MIP-1 α , which confirmed an essential role of tryptophan metabolism in exaggerated cytokine release upon SARS-CoV-2 infection (**Fig. 4c, Extended Data Fig. 7**). In addition, direct inhibition of purine metabolism with mycophenolic acid (MPA), which blocks the rate-limiting enzyme inosine monophosphate dehydrogenase (IMPDH) in *de novo* synthesis of guanosine nucleotides, significantly reduced levels of IL-10, IFN- γ , IL-15, IL-12 p40, IL-17A and TNF- α induced by SARS-CoV-2 infection. However, a profound increase in proinflammation cytokines of IL-6, GM-CSF, IL-1 α and IL-1 β was also observed, which suggests the exacerbated hyperinflammation upon interfering with purine metabolism (**Fig. 4d, Extended Data Fig. 8**). Finally, we evaluated the effect of interfering these metabolic pathways on the SARS-CoV-2 replication in PBMCs. Our results showed that arginine supplementation, IDO1 or IMPDH inhibitors did not affect the SARS-CoV-2 replication (**Extended Data Fig. 9**). Taken together, these data suggest that targeting dysregulated host metabolism may serve as a viable approach to suppressing SARS-CoV-2-induced inflammatory cytokine secretion.

Discussion

CRS has been reported contribute to vascular damage, immunopathology, and adverse clinical outcomes in COVID-19 patients^{2,4,7}. Hence, strategies to constrain the proinflammation cytokines release are emerging as potential therapies for COVID-19^{11,34}. Increasing studies have trialed strategies, including monoclonal antibodies targeting inflammatory cytokines or small-molecule inhibiting the upstream or downstream regulatory pathways, to dampen the inflammatory responses^{11,34}. However, better understanding the driving causes of cytokine storm and identifying potential multi-cytokine blockers are still urgent need. Considering the previously reported high correlation between metabolism and immune response^{13,17,27,35}, and the key role of metabolism in regulating cytokine release upon viral infection^{16,22}, we speculated that the metabolism and CRS may correlate well and correcting the dysregulated host metabolism may suppress the SARS-CoV-2-induced CRS. We thus characterized the metabolic and immune profiling in the same COVID-19 patient cohort, and our network correlation analysis between circulating metabolite and cytokine levels revealed the potential regulatory role of arginine metabolism, tryptophan metabolism, and purine metabolism in proinflammatory responses.

The cytokine profiling of COVID-19 patients in our study provide further evidence that most CRS-associated cytokines, such as IL-6, IL-1 β , IL-10, IL-18 and IFN- γ , are dramatically elevated in severe patients. Conversely, the increased inflammatory responses

287 experienced progressive reduction accompanying with clinical recovery in mild COVID-
288 19 patients during hospitalization. These findings highlight the need for novel therapies to
289 block multiple cytokines linked to CRS. Emerging evidence suggests the key role of
290 metabolism in regulating cytokine secretion ^{13,15,21}. For example, choline uptake and
291 metabolism modulate the IL-1 β and IL-18 production in stimulated macrophages ³⁶. α -
292 ketoglutarate-supplemented diet has been reported to induce IL-10 production, thus leading
293 to suppression of chronic inflammation and extension of life span ³⁷. Particularly, elevated
294 glucose levels in COVID-19 patients promote SARS-CoV-2 replication and cytokine
295 production in monocytes ¹⁶. A very recent study suggests that the a high kynurenic acid to
296 kynurenine ratio is linked to immune responses and clinical outcomes in male COVID-19
297 patients ³⁸. In addition, Vitamin D deficiency associated with the uncontrolled cytokine
298 production and disease severity of COVID-19 ^{39,40}, emphasizing the need of Vitamin D
299 supplementation for COVID-19 treatment.

300 Our metabolomics data identified alterations of circulating metabolite levels in patient's
301 serum and determined dysregulated metabolic pathways upon SARS-CoV-2 infection.
302 Consistent with recent studies ^{18,41}, the metabolites that are associated with arginine
303 metabolism, tryptophan metabolism, TCA cycle as well as purine and pyrimidine
304 metabolism changed remarkably. Interestingly, correlation network analysis between
305 metabolites and cytokines in mild and severe patients revealed that circulating cytokine
306 levels were highly correlated with arginine metabolism, tryptophan metabolism, nucleic

acid metabolism and energy metabolism. Moreover, our time-series clustering analysis in mild patients identified four distinct clusters of longitudinal trajectories delineating the crosstalk between metabolism and inflammatory response. These results suggest perturbation of metabolic pathways may partially contribute to the consequential CRS in COVID-19.

Arginine is a conditionally essential amino acid for adult mammals and involves in immune dysfunctions during viral infection^{15,21}. Intriguingly, we observed that circulating levels of arginine had a significant negative correlation with CRS-related proinflammatory cytokines, and supplementation of arginine markedly inhibited the elevation of proinflammatory cytokines upon SARS-CoV-2 infection, confirming its role as a potent metabolic regulator. In addition, manipulation of tryptophan metabolism led to marked decline in proinflammatory cytokines. Recent studies identified the important role of IDO1-kynurenine/kynurenic acid-arylhydrocarbon receptors (AhRs) signaling in inflammation and multiple organ injuries in SARS-CoV-2 infection^{38,42}, which is consistent with our findings. We also showed that inhibition of purine metabolism exacerbated inflammatory response. However, the inhibition of pyrimidine biosynthesis pathway reportedly arrested SARS-CoV-2 replication and suppressed inflammatory cytokines production⁴³. These results suggested the importance of the balance between purine and pyrimidine metabolism in viral replication and immune response. Indeed, combined agents targeting multiple pathogenic factors involved in the hyperinflammation

is emerging as the way forward for supportive care for COVID-19³⁴. It is therefore possible that cocktails of drugs targeting multiple metabolic pathways for global cytokine blockade might constitute a new class of therapeutic strategy.

Due to the resource restriction, our study validated the effects of supplemented metabolites or pharmacological inhibitors in regulating the CRS induced by SARS-CoV-2 infection using the *ex-vivo* model of PBMCs isolated from the infected rhesus macaques. Although the isolated PBMCs *ex vivo* models has been extensively used for the evaluation of cytokine release^{44,45}, it would have been ideal to perform such analyses in rhesus macaque *in vivo* models. We tested the impacts of metabolism intervening on the immunological responses in the heterogenous PBMCs. Yet, given that SARS-CoV-2 infection reduces innate antiviral defenses while activates inflammatory cytokine release⁴⁶⁻⁴⁸, analyses on sorted subpopulations of immune cells would help to more precisely understand the roles of metabolism in regulating the release of specific cytokines with discriminating functions, proinflammatory or suppressive, from different immune cell types.

In summary, our study performed a comprehensive metabolic and immune profiling in COVID-19 patients and showed that reprogrammed host metabolism was tightly linked to the burst of proinflammatory cytokines. Beyond providing a comprehensive resource of metabolism and immunology data to support further investigation of COVID-19, our study also uncovered new insights related to tight correlation between metabolism and cytokine

346 release, and thereby provided potential therapeutic strategy for treatment of fatal CRS
347 induced by SARS-CoV-2 infection.

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Materials and methods

Patients and samples

A total of 44 COVID-19 patients and 17 healthy controls were enrolled in this study. Cross-sectional serum samples from 37 COVID-19 patients were collected from Chongqing Three Gorges Central Hospital, Chongqing Public Health Medical Center and Yongchuan Hospital Affiliated to Chongqing Medical University. Sequential serum samples from 7 mild patients were collected from Yongchuan Hospital Affiliated to Chongqing Medical University with intervals of 3 days. Serum samples from 17 healthy controls were collected from The Second and Third Affiliated Hospital of Chongqing Medical University. Patients were confirmed to be infected with SARS-CoV-2 by RT-PCR assays (DAAN Gene) on nasal and pharyngeal swab specimens. Briefly, two target genes, including open reading frame1ab (ORF1ab) and nucleocapsid protein (N), were simultaneously amplified and tested during RT-PCR. Primers of RT-PCR testing for SARS-CoV-2 were designed according to the recommendation by the Chinese CDC (ORF1ab-forward: CCCTGTGGGTTTTACACTTAA, ORF1ab-reverse: ACGATTGTGCATCAGCTGA, ORF1ab-probe: 5'-CCGTCTGCGGTATGTGGAAAGGTTATGG-3' (FAM dye labeled); N-forward: GGGGAAGTTCTCCTGCTAGAAT, N-reverse: CAGACATTTTGCTCTCAAGCTG, N-probe: 5'-TTGCTGCTGCTTGACAGATT-3' (VIC dye labeled)). PCR cycling: 50°C for 15 min, 95 °C for 15 min, 45 cycles containing

94°C for 15 s, 55°C for 45 s (fluorescence collection). Ct values less than 37 and greater than 40 were defined as positive and negative, respectively, for both genes.

Detection of IgG and IgM against SARS-CoV-2

All serum samples were inactivated at 56 °C for 30 min and stored at -20 °C before testing. IgG and IgM against SARS-CoV-2 in plasma samples were tested using magnetic chemiluminescence enzyme immunoassay kits supplied by Bioscience Co. (approved by the China National Medical Products Administration; approval numbers 20203400183(IgG) and 20203400182(IgM)), according to the manufacturer's instructions. Antibody levels are presented as the measured chemiluminescence values divided by the cutoff (S/CO).

Serum sample cytokine measurement

Concentrations of 48 cytokines and chemokines in each serum sample were measured using the Bio-Plex Human Cytokine Screening Panel (48-Plex no. 12007283, Bio-Rad) on a Luminex 200 (Luminex Multiplexing Instrument, Merck Millipore) following the manufacturer's instructions.

Clinical data collection

Epidemiologic, demographic, clinical presentations, laboratory tests, treatment and outcome data were collected from inpatient medical records. Laboratory data collected for each patient included complete blood count, coagulation profile, serum biochemical tests

(including renal and liver function, electrolytes, lactate dehydrogenase and creatine kinase), serum ferritin and biomarkers of infection.

Clinical Definitions

Clinical classification was defined based on the COVID-19 Treatment Guidelines (National Health Commission of the People's Republic of China). A confirmed case of SARS-CoV-2 infection was defined as an individual with nasopharyngeal swabs positive for SARS-CoV-2 nucleic acid by RT-PCR as described above. Severe COVID-19 cases were whom meeting any of the following criteria: 1) respiratory distress (≥ 30 times/minutes), 2) the oxygen saturation $\leq 93\%$ at rest, 3) the arterial partial pressure of oxygen (PaO_2) / the fraction of inspired oxygen (FiO_2) ≤ 300 mmHg. Mild patients were defined as COVID-19 patients with symptoms but could not be classified as severe. Symptoms onset date was defined as the date on which symptoms first began. Symptoms included fever, fatigue, dry cough, inappetence, myalgia, dyspnea, expectoration, pharyngalgia, diarrhea, nausea, dizziness, headache, abdominal pain, chill, rhinorrhea, chest stuffiness or nasal congestion.

Ethical approval

The study was approved by the Ethics Commission of Chongqing Medical University (ref. no. 2020003). Written informed consent was waived by the Ethics Commission of the designated hospitals for emerging infectious diseases.

Sample preparation for metabolomics

Human serum samples, 20 μ L each, were heated at 56 °C for 30 min followed by adding 60 μ L ethanol to inactive SARS-COV-2 virus. The suspension was evaporated to dryness using a SpeedVac concentrator (Thermo Scientific). Metabolites from the serum pellet were extracted with 540 μ L 80% methanol in water, followed by vigorous vortex and cooled centrifuge at 4 °C. Then, equal aliquots of the supernatant from each sample (20 μ L) were pooled together to make the quality control (QC) samples. The remaining supernatant was divided into two fractions, one for targeted metabolomics and the other for untargeted metabolomics analysis. All the samples were evaporated to dryness.

Targeted metabolomics

For targeted metabolomics, dried metabolites were reconstituted in LC-MS grade water with 0.03% formic acid, vortex-mixed and centrifuged at 4°C for 15 min to remove debris. Samples were randomized and blinded before analyzing by LC-MS/MS.

Chromatographic separation was performed on a Nexera Ultra High-Performance Liquid Chromatograph (UHPLC) system (Shimadzu), with a RP-UPLC column (HSS T3, 2.1 mm $\times$ 150 mm, 1.8 μ m, Waters) and the following gradient: 0-3 min 99% A; 3-15 min 99-1% A; 15-17 min 1% A; 17-17.1 min 1-99% A; 17.1-20 min 99% A. Mobile Phase A was 0.03% formic acid in water. Mobile Phase B was 0.03% formic acid in acetonitrile. The flow rate was 0.25 mL $\cdot$ min⁻¹, the column was at 35°C and the autosampler was at 4°C. Mass data

acquisition was performed using an AB QTRAP 6500+ triple quadrupole mass spectrometer (SCIEX, Framingham, MA) in multiple reaction monitoring (MRM) mode for the detection of 258 unique endogenous water-soluble metabolites as previously described, with some modifications^{15,49}. Chromatogram review and peak area integration were performed using MultiQuant 3.0.2 (SCIEX, Framingham, MA).

Untargeted metabolomics

For untargeted metabolomics, dried samples were reconstituted in acetonitrile/water mixture (v/v, 1:1). After vortex, samples were centrifuged at 4°C for 15 min to remove debris. Samples were randomized and blinded before LC-MS/MS analysis.

Chromatographic separation was performed on an Agilent 1290 infinity II LC system, with an Agilent Eclipse Plus C18 column (2.1 mm × 100 mm, 1.8 µm). The gradient was set as follows: 0-2 min 95% A; 2-20 min 95-0% A; 20-25 min 0% A; post-run time for equilibration, 5 min in 95% A. Water containing 0.1% formic acid and acetonitrile containing 0.1% formic acid acted as the mobile phase A and B in the positive-ion mode of mass spectrometry analysis. While in the negative-ion mode of mass spectrometry analysis, the 0.1% formic acid was replaced with 1 mM ammonium fluoride. The flow rate was set as 0.3 mL·min⁻¹ and the temperatures of the column and autosampler were set as 40°C and 4°C separately. The data acquisition was performed on a 6546 Q-TOF mass spectrometry equipped with a dual electrospray (ESI) ion source (Agilent Technologies,

Santa Clara, CA). The optimized ESI Q-TOF parameters were set as follows. The temperature and flow rate of sheath gas were 350°C and 11 L·min⁻¹. The voltages of capillary, fragmentor and skimmer were set as 4000 V, 140 V and 65 V, respectively. The spectra were internally mass calibrated in real time by continuous infusion of a reference mass solution using an isocratic pump connected to a dual sprayer feeding into an ESI source. MassHunter Acquisition software (Agilent Technologies, Santa Clara, CA) was employed to perform data acquisition.

The Agilent Masshunter Workstation (Profunder software, version 10.0) was firstly utilized to filter the candidate metabolites. The metabolites were identified based on their accurate mass, retention time, isotopic patterns. And the qualitative analysis software (version 10.0) was employed to check the MS/MS spectra of the identified metabolites to avoid false results. Further, to expand the qualitative coverage of specific metabolic pathways, the Agilent Pathway to PCDL software was employed to perform targeted extraction of metabolites from the raw data. The peak areas of the identified metabolites and the other metabolite features which can be consistently detected from 80% samples were achieved from the Profunder software.

Normalization and integration of targeted and untargeted metabolomics data

For both targeted and untargeted metabolomics, quality control (QC) samples composed of an equal aliquot of all test samples were prepared and inserted in an interval of ten test

572 samples to monitor the stability of instrument and normalize the variations during the run.
573 This served as an additional quality control measure of analytical performance and a
574 reference for normalizing raw metabolomic data across samples.

575 To remove potential inter-batch variations, the mean peak area of each metabolite from all
576 the QC samples in a given batch (QC_{all}), as well as the mean peak area of each metabolite
577 from the QC samples that are the most adjacent to a given group of test samples (QC_{adj})
578 were first calculated. The ratio between these two mean peak areas for each metabolite was
579 computed by dividing the same QC_{all} by each QC_{adj} and used as the normalization factor
580 for each given group of test samples. The peak area of each metabolite from each test
581 sample were normalized by multiplying their corresponding normalization ratio to obtain
582 the normalized peak areas. In addition, to effectively correct the sample to sample variation
583 in biomass that may contribute to systematic differences in metabolite abundance detected
584 by LC-MS, we generated the scaled data by comparing the normalized peak area of each
585 metabolite to the sum of the normalized peak area from all the detected (for targeted
586 metabolomics) or identified metabolites (for untargeted metabolomics) in that given
587 sample.

588 Our validation analyses suggested that these normalization and scaling steps could
589 effectively correct both the inter-sample artificial differences in sample biomass and inter-
590 batch systematic variations in detected metabolite abundance.

For metabolites that were detected in both targeted and untargeted methods, data from targeted method were used for the subsequent analysis, except for the data of carnitine metabolites which are from untargeted methods. The final metabolomic data matrix (102 rows of patients/controls and 253 columns of metabolites) used for the downstream analyses or modeling is included in **Table S2**.

Virus preparation

Viral stocks of SARS-CoV-2 were obtained from the Center of Diseases Control, Guangdong Province China. Virus were amplified on Vero E6 cells and concentrated by ultrafilter system via 300 kDa module (Millipore). Vero E6 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal bovine serum (FBS).

Isolation of PBMCs from monkey peripheral blood

Peripheral blood mononuclear cells (PBMCs) were isolated from monkey peripheral blood using Ficoll-PaqueTM (Sigma-Aldrich). Peripheral blood sample (4 mL) from each mock-infected and SARS-CoV-2-infected Rhesus Macaques was drawn into vacutainer tubes. The ficoll density gradient centrifugation method was used to separate the PBMCs. We diluted the blood with 1× phosphate-buffered saline (PBS) 1:1 and then transferred it to the ficoll tube. After centrifugation at 1000× g for 20 min, the buffy coat of PBMCs was pooled

and transferred into a 15 mL falcon. PBMCs were then washed twice with 10 mL PBS and centrifuged at 250× g for 10 min. The cell pellets were resuspended in RPMI 1640 medium.

Antiviral assays

PBMCs (2×10^4 cells / well) isolated from mock-infected rhesus macaques were seeded in 24-well plates and infected with mock or SARS-CoV-2 at MOI 0.05. Virus was added together with drugs and incubated in RPMI 1640 medium supplemented with 10% FBS with different drugs. Viral loads of SARS-CoV-2 in PBMCs were assessed 24 h after infection.

Viral RNA measurements

SARS-CoV-2 RNA from PBMCs was isolated using the Direct-zol™ RNA MiniPrep (Zymo Research Corp) according to the manufacturer's instructions. Briefly, 50 µL DNase/RNase-Free Water was used to elute RNA. Real time RT-PCR was used to quantify viral genome in samples using TaqMan Fast Virus One-Step Master Mix (Thermo Fisher) and purified viral RNA of SARS-CoV-2 as a standard curve, performed on CFX384 Touch Real-Time PCR Detection System (Bio-Rad). Conditions for RT-PCR were used as follows: 25°C for 2 min, 50°C for 15 min, 95°C for 2 min, then 40 cycles at 95°C 5 sec and 60°C 31 sec. Primers and probe, specific for SARS-CoV-2 *NP* gene was synthesized according to sequences reported by China Centers for Disease Control (CDC), Target-2-F:

627 GGGGAAGTTCTCCTGCTAGAAT, Target-2-R: CAGACATTTTGCTCTCAAGCTG,
628 Target-2-P: 5'-FAM- TTGCTGCTGCTTGACAGATT-TAMRA-3'.

629 **Measurement of cytokines production by PBMCs**

630 PBMCs (1×10^6 cells/well) isolated from mock-infected and SARS-CoV-2-infected
631 Rhesus Macaques were seeded in 96-well plates and treated with different drugs. For
632 cytokine measurement of PBMCs culture supernatant samples, MILLIPLEX MAP Non-
633 Human Primate Cytokine Magnetic Bead Panel-Immunology Multiplex
634 Assay (PRCYTOMAG-40K, Millipore USA) was used according to the manufacturer's
635 protocol, which was performed on Bio-plex machine. Inflammatory cytokines in this panel
636 included IL-1 β , IL-4, IL-5, IL-6, IL-8/CXCL8, G-CSF, GM-CSF, IFN- γ , IL-1RA, IL-2, IL-
637 10, IL-12 p40, IL-13, IL-15, IL-17A/CTLA8, MCP-1/CCL2, MIP-1 β /CCL4, MIP-
638 1 α /CCL3, sCD40L, TGF- α , TNF- α , VEGF and IL-18.

639 **Animals and experimental procedures**

640 For SARS-CoV-2 virus infections, we inoculated rhesus macaques with total 5 mL of 10^6
641 pfu/mL SARS-CoV-2 intratracheally (2.5 mL) and intranasally (2.5 mL). All animal
642 experiments were performed according to protocols approved by the Institutional Animal
643 Care and Use Committee of Institute of Medical Biology, Chinese Academy of Medical
644 Science, and performed in the ABSL-4 facility of Kunming National High-level Biosafety
645 Primate Research Center, Yunnan China.

Partial least squares discrimination analysis (PLS-DA)

PLS-DA was performed on normalized metabolomics data using SIMCA-P software (version 14.1, Umetrics, Umea, Sweden) and unit variance (UV) scaling was utilized before multivariate analysis.

t-distributed stochastic neighbor embedding (t-SNE) dimensionality reduction

t-SNE scatterplots were generated of log10-transformed normalized metabolomics data using R Rtsne (v0.15) package with perpiexity of 5 and theta of 0.01.

Longitudinal trajectory analysis of serum metabolites and cytokines

To estimate serum cytokine and metabolite longitudinal trajectories of follow-up mild COVID-19 patients, generalized additive model (GAM) adjusted for age and gender was fitted for each cytokine and metabolite. GAM was performed by R mgcv (v1.8-31) package with default parameters.

Mann-Whitney U tests and Benjamini-Hochberg multiple comparison test were carried out at each time point to identify significant altered serum cytokines and metabolites compared with healthy controls (base lines). Cytokines and metabolites with FDR < 0.05 were considered significant. Based on the relative abundance of significant metabolites and cytokines after z-score scaled, fuzzy c-mean clustering was performed using R Mfuzz (v2.44.0) package, and parameter m was set to 1.5.

Correlation analysis of serum metabolites and cytokines

Linear regressions models adjusted for gender and age were used to estimate correlations between serum metabolites and cytokines, as log10-transformed, in mild and severe patients or follow-up patients. Linear regressions models conducted in R using the lm base function. For mild and severe patients, cytokines and metabolites which were significantly altered in severe patients were applied to linear regression models. For follow-up patients, metabolites and core CRS-related cytokines involved in four clusters were applied to linear regression models. *P* values were corrected using Benjamini-Hochberg multiple comparison test and correlations with FDR < 0.05 were considered significant and used for subsequent analysis.

Then, based on the significant cytokine-metabolite correlations in serum of mild and severe patients, weighted, undirected correlation networks were built by R igraph (v1.2.5) package and clusters were determined based on fast greedy modularity optimization algorithm. Molecule types and correlation directions were color coding.

KEGG pathway analysis

Serum metabolites identified by targeted and untargeted metabolomics were integrated for KEGG pathway analysis. KEGG metabolic pathways and involved metabolites were downloaded through KEGG API (<https://www.kegg.jp/kegg/rest/keggapi.html>). Significant enriched KEGG pathways based on given serum metabolites were determined

by R clusterProfiler (v3.12.0) package with Benjamini-Hochberg multiple comparison test. For significantly altered serum metabolites in mild and severe patients, pathways with FDR < 0.1 were considered significant. For metabolites significantly associated with cytokines in mild and severe patients, pathways with FDR < 0.1 and enriched for at least 3 cytokines were considered enriched. For serum metabolites in each trajectory cluster in follow-up patients, pathways with FDR < 0.05 were considered enriched.

For metabolites with progressive change in mild and severe patients, metabolites which increased or decreased in mild patients (compared with healthy controls) and severe patients (compared with mild patients) and showed significance in severe patients were considered consistently altered. Metabolic pathways with FDR < 0.1 were considered significantly enriched.

Statistical analysis

Quantification methods and statistical analysis methods for metabolite and cytokine analyses were mainly described and referenced in the respective Method Details subsections.

Additionally, Serum metabolites and cytokines with significant altered abundance were identified by Mann-Whitney U tests and Benjamini-Hochberg multiple comparison test, which were performed by R stat (v3.6.0) package. Serum cytokines and metabolites with FDR < 0.05 were considered significant and used for subsequent analysis. Statistical

significance of cytokine and viral RNA abundance in cell culture supernatant between different conditions were considered using one-way ANOVA followed by Benjamini-Hochberg multiple comparison test, which were performed by GraphPad prism (v8.2.1).

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Author contributions: N.X., M.N. and Z.H. conceived the project, designed the study and wrote the paper. N.X. designed and performed the metabolite-cytokine correlation analyses and interpreted the results. M.N. and K.L. designed and performed the experiments related to PBMCs derived from rhesus macaques. H.P. and B.W. performed the metabolomics experiments. X.M. and X.R. assisted in the metabolomics experiments. T.J., P.Z., W.J., Q.Y., and C.Z. provided the clinical samples and information. J.H., Q.L. and N.T. performed laboratory and cytokine tests. A.H. and Z.H. acquired the fundings. Z.H. supervised the project.

Competing interests: Authors declare no competing interests.

Figure legends

Fig. 1 | Study design and metabolic profiling in serum samples from mild and severe COVID-19 patients

a, Overview of cohort (including mild (n = 21) and severe (n = 23) COVID-19 patients and healthy controls (n = 17)) and the study design. **b**, t-SNE plot distributed healthy controls (n = 17), mild patients (n = 14) and severe patients (n = 23) according to serum metabolites detected from targeted and untargeted metabolomics. **c-d**, Volcano plots comparing serum metabolites between mild (**c**) or severe (**d**) patients with healthy controls. Significantly increased and decreased metabolites are shown in red and blue, respectively, and metabolites with no statistical significance are shown in gray. Top 5 most significantly increased or decreased metabolites in each group are labeled. **e**, KEGG metabolic pathways in mild and severe patients enriched by significantly changed serum metabolites in **c** and **d**. **f**, Schematic depicting the key metabolic pathways altered in response to SARS-CoV-2 infection. Gray nodes represent metabolites that were not tested. Metabolite alteration are represented by color intensity, and borders are color-coded by statistical significance. Statistical significance was assessed using two-sided Mann-Whitney U test (**c-d**), and Fisher's exact test (**e**) followed by Benjamini-Hochberg multiple comparison test.

Fig. 2 | Metabolite-cytokine correlation in serum samples from COVID-19 patients

a, Metabolic pathways enriched by metabolites that are significantly correlated with each

presented cytokine. T statistics and statistical significance of metabolite-cytokine correlations were assessed based on two-sided t tests of regression coefficients followed by Benjamini-Hochberg multiple comparison test. Mean absolute (abs.) T statistics of metabolites in each pathway were calculated and represented by color intensity. Statistical significance of pathway enrichment analysis was assessed by Fisher's exact test and followed by Benjamini-Hochberg multiple comparison test. **b-e**, Correlation networks of key CRS-related cytokines and metabolites significantly altered in severe patients. Nodes are color-coded by molecule types and metabolic pathways. Edges are color-coded by association directions. Networks were clustered by fast greedy modularity optimization algorithm. **f-h**, Chord diagrams of significant metabolite-cytokine correlations with metabolites involved in arginine metabolism (**f**), purine metabolism (**g**), tryptophan and NAD^+ metabolism (**h**), respectively. Chords are color-coded by association directions consistent with **b-e**.

Fig. 3 | Longitudinal trajectories and metabolite-cytokine correlation in mild COVID-19 patients

a, Longitudinal trajectory clustering of significantly changed serum metabolites, cytokines in follow-up patients with mild COVID-19. Metabolite and cytokine abundance in healthy controls were used as base line. Black lines represent the average trajectory for each cluster. **b**, Heatmap comparison of cytokines at distinct time point in follow-up patients. Log2 fold change of mean cytokine abundance in each interval relative to healthy controls is

represented by color intensity. **c**, Enriched KEGG metabolic pathways in each cluster. **d**, Serum metabolite trajectories based on normalized data in follow-up patients. Blue solid lines pass through the mean of each measurement at the specific time interval, and dotted lines represent the mean of measurements in healthy controls. Generalized additive model (GAM) regression lines were represented by the black solid lines, with 95% confidence intervals for the regression line denoted by gray filled areas. Data are presented as mean $\pm$ SEM. with individual data points shown. **e**, Chord diagrams of significant associations between metabolites and core CRS-related cytokines in cluster 1 (left), cluster 2 (middle), cluster 3 and 4 (right), respectively. Statistical significance was assessed by Fisher's exact test (**c**) and linear regression model adjusted for age and gender (**e**) followed by Benjamini-Hochberg multiple comparison test.

Fig. 4 | Targeting metabolism modulates cytokine release in PBMCs *ex vivo* model

a, Schematic representation of the experimental workflow. PBMCs, isolated from peripheral blood of mock-infected or SARS-CoV-2-infected rhesus macaques, were seeded in 96-well plates with vehicle or different drugs dissolved in the medium. 24 hours post-seeding, cytokine abundance in cell culture was quantified. **b-d**, Metabolism diagrams and level of indicated cytokines and chemokines measured 24 h after supplementation of 1.25 mM arginine (**b**), 0.1 mM IDO1 inhibitor Epacadostat (**c**), and 0.1 mM IMPDH inhibitor Mycophenolic acid (MPA, **d**) in PBMCs. Data are presented as mean $\pm$ SEM. with individual data points shown. Statistical significance was assessed using one-way ANOVA

followed by Benjamini-Hochberg multiple comparison test.

Extended Data Fig. 1 | Clinical information of mild and severe COVID-19 patients

Clinical information of healthy controls, mild and severe COVID-19 patients. Data are presented as mean $\pm$ SEM. with individual data points shown. Statistical significance was assessed using either one-way ANOVA followed by Benjamini-Hochberg multiple comparison test or unpaired, two-sided *t*-test.

Extended Data Fig. 2 | Overview of metabolomics data in COVID-19 patients

a, General workflow of metabolomic profiling experiments and data analysis. **b-c**, Partial Least Squares Discriminant Analysis (PLS-DA) of targeted metabolomics data (**b**) and untargeted metabolomics data (**c**) of healthy controls, mild and severe COVID-19 patients. **d**, Venn diagram depicting the number of significantly altered serum metabolites in each group after integrating targeted and untargeted metabolomics data. **e**, Metabolic pathways enriched based on metabolites progressively increased or decreased in mild and severe patients compared with healthy controls. Statistical significance was assessed by Fisher's exact test and followed by Benjamini-Hochberg multiple comparison test. **f-i**, Line charts of metabolites which significantly altered in severe patients involved in arginine metabolism (**f**), TCA cycle (**g**), tryptophan metabolism (**h**), NAD⁺ metabolism (**i**).

Extended Data Fig. 3 | Overview of cytokine data in COVID-19 patients

a, Alterations in serum cytokine abundance in mild and severe patients compared with healthy controls. Log2 fold change is represented by color intensity. Shape represents alteration significance, and FDR is size-coded. **b**, Violin plots comparing serum cytokines abundance in healthy controls, mild patients and severe patients. Data are presented as mean and quantiles with individual data points shown. Statistical significance was assessed using two-sided Mann-Whitney U test followed by Benjamini-Hochberg multiple comparison test.

Extended Data Fig. 4 | Longitudinal cytokine trajectories in follow-up mild COVID-19 patients

a, Schematic diagram of sample collection for follow-up patients. Blue squares indicate the time of serum collection; red circles represent the time of symptoms onset; dark blue lines represent the time of hospitalization. **b-c**, Longitudinal antibodies (**b**), log10 transformed cytokine and chemokine concentration (**c**) trajectories in an interval of 3 days. Blue solid lines pass through the mean of each measurement at the specific time interval, and dotted lines represent the mean of measurements in healthy controls. GAM regression lines are indicated by the black solid lines, with 95% confidence intervals for the regression lines denoted by gray filled areas. Data are presented as mean $\pm$ SEM. with individual data points shown.

Extended Data Fig. 5 | Longitudinal metabolite trajectories in follow-up mild COVID-

19 patients

a, Heatmap comparison of metabolites at distinct time point in follow-up patients in each cluster. Log2 fold change of metabolite abundance in each interval relative to healthy controls is represented by color intensity. **b**, Serum metabolite trajectories based on normalized data in follow-up patients. Blue solid lines pass through the mean of each measurement at the specific time interval, and dotted lines represent the mean of measurements in healthy controls. GAM regression lines are represented by the black solid lines, with 95% confidence intervals for the regression denoted by gray filled areas. Data are presented as mean $\pm$ SEM. with individual data points shown.

Extended Data Fig. 6 | Cytokine abundance after supplementation with arginine

Levels of indicated cytokines and chemokines measured 24 h after supplementation of 1.25 mM arginine in isolated PBMCs derived from mock-infected or SARS-CoV-2-infected rhesus macaques. Data are presented as mean $\pm$ SEM. with individual data points shown. Statistical significance was assessed using one-way ANOVA followed by Benjamini-Hochberg multiple comparison test.

Extended Data Fig. 7 | Cytokine abundance after treatment with IDO1 inhibitor

a, Ratio of kynurenine (Kyn) to tryptophan (Try) in healthy controls and COVID-19 patients. **b**, Levels of indicated cytokines and chemokines measured 24 h after 0.1 mM

IDO1 inhibitor Epacadostat treatment in isolated PBMCs derived from mock-infected or SARS-CoV-2-infected rhesus macaques. Data are presented as mean $\pm$ SEM with individual data points shown. Statistical significance was assessed using one-way ANOVA followed by Benjamini-Hochberg multiple comparison test.

Extended Data Fig. 8 | Cytokine abundance after treatment with IMPDH inhibitor

Levels of indicated cytokines and chemokines measured 24 h after treatment of 0.1 mM IMPDH inhibitor mycophenolic acid (MPA) in isolated PBMCs derived from mock-infected or SARS-CoV-2-infected rhesus macaques. Data are presented as mean $\pm$ SEM. with individual data points shown. Statistical significance was assessed using one-way ANOVA followed by Benjamini-Hochberg multiple comparison test.

Extended Data Fig. 9 | Viral RNA quantification in isolated PBMCs *ex vivo*

a, PBMCs were isolated from uninfected rhesus macaque and were either mock or SARS-CoV-2 infected and cultured for 24 h under different treatment conditions. Viral RNA in PBMCs were then assessed by quantitative PCR analysis. **b**, Levels of SARS-CoV-2 RNA in PBMCs quantified 24 h after infected and supplementation of 1.25 mM arginine, 0.1 mM IDO1 inhibitor Epacadostat, and 0.1mM IMPDH inhibitor Mycophenolic acid (MPA), respectively. Statistical significance was assessed using one-way ANOVA followed by Benjamini-Hochberg multiple comparison test. Data are presented as mean $\pm$ SEM. with individual data points shown.

854 **Supplementary Tables**

855 **Extended Data Table 1 | Clinical characteristics of healthy controls and COVID-19**
856 **patients**

857 **Extended Data Table 2 | Normalized targeted and untargeted metabolomics data**

Figure 1

Figure 2

Figure 3

Figure 4

a

b

c

d

Extended Data Figure 1

Extended Data Figure 2

Extended Data Figure 3

Extended Data Figure 4

a

Extended Data Figure 6

Extended Data Figure 7

Extended Data Figure 8

Extended Data Figure 9

Extended Data Table 1 | Clinical characteristics of healthy controls and COVID-19 patients

	Healthy Controls	COVID-19 Patients		
Variables	Healthy Controls (n = 17)	Mild (n = 14)	Severe (n = 23)	Follow-up (n = 7)
Sex - no.^a (%)				
Male	8 (47.1%)	8 (57.1%)	9 (39.1%)	4 (57.1%)
Female	9 (52.9%)	6 (42.9%)	14 (60.9%)	3 (42.9%)
Age - year				
Mean $\pm$ SD. ^b	47.5 $\pm$ 13.8	34.2 $\pm$ 19.0	60.8 $\pm$ 16.7	41.9 $\pm$ 19.3
Median (IQR) ^c	50 (40-54)	38 (20-48)	65 (52-78)	46 (28-56)
Range	20-72	0.66-67	31-80	16-63
BMI, kg/m²				
Median (IQR)	25.7 (24.7-27.4)	23.4 (20.7-26.1)	24.5 (23.0-27.2)	-
Time from Onset to Admission, days				
Median (IQR)	-	6 (4-8)	7 (5-8)	2 (1-3)
Symptoms - no. (%)				
Fever	-	11 (78.6%)	13 (56.5%)	5 (71.4%)
Fatigue	-	1 (7.1%)	12 (52.2%)	2 (28.6%)
Dry cough	-	8 (57.1%)	12 (52.2%)	3 (42.9%)
Inappetence	-	3 (21.4%)	13 (56.5%)	-
Myalgia	-	1 (7.1%)	3 (13.0%)	2 (28.6%)
Dyspnea	-	-	9 (39.1%)	-
Expectoration	-	2 (14.3%)	9 (39.1%)	-
Pharyngalgia	-	2 (14.3%)	1 (4.3%)	1 (14.3%)
Diarrhea	-	3 (21.4%)	2 (8.7%)	-
Nausea	-	-	3 (13.0%)	-
Dizziness	-	-	3 (13.0%)	-
Headache	-	1 (7.1%)	2 (8.7%)	-
Abdominal pain	-	-	2 (8.7%)	-
Chill	-	1 (7.1%)	4 (17.4%)	-
Rhinorrhea	-	3 (21.4%)	3 (13.0%)	-
Chest stuffiness	-	3 (21.4%)	9 (39.1%)	-
Nasal congestion	-	1 (7.1%)	2 (8.7%)	-
Comorbidity- no. (%)				
Hypertension	-	-	3 (13.0%)	1 (14.3%)

Cardiovascular disease	-	-	1 (4.3%)	-
Diabetes	-	-	2 (8.7%)	-
COPD	-	-	1 (4.3%)	-
Chronic liver disease	-	1 (7.1%)	1 (4.3%)	-
Oxygenation Index - mmHg				
Median (IQR)	-	390 (362-395)	276 (160-340)	429 (384-450)
Treatment - no. (%)				
Kaletra	-	13 (92.9%)	23 (100.0%)	7 (100.0%)
Oseltamivir	-	1 (7.1%)	3 (13.0%)	-
Interferon therapy	-	14 (100.0%)	23 (100.0%)	7 (100.0%)
Antibiotic therapy	-	1 (7.1%)	18 (78.3%)	2 (28.6%)
Glucocorticoid	-	-	10 (43.5%)	2 (28.6%)
Chinese medicine therapy	-	3 (21.4%)	17 (73.9%)	7 (100.0%)
Oxygen therapy	-	-	9 (39.1%)	7 (100.0%)
Mechanical ventilation	-	13 (92.9%)	23 (100.0%)	-
Blood routine				
White blood cell count, $\times 10^9/L$	-	-	9.6 (9.3-13.8)	4.7 (4.3-6.1)
Red blood cell count, $\times 10^9/L$	-	-	4.1 (3.8-4.1)	4.9 (4.4-5.3)
Neutrophils count, $\times 10^9/L$	-	2.7 (2.1-3.2)	3.4 (3.0-4.9)	2.7 (2.4-3.7)
Lymphocytes count, $\times 10^9/L$	-	1.5 (1.2-2.1)	0.8 (0.6-1.1)	1.2 (1.1-1.4)
Platelets count, $\times 10^9/L$	-	-	259.0 (163.0-356.0)	179 (158-185.5)
Haemoglobin, g/L	-	30.8 (30.3-31.5)	29.6 (28.8-30.7)	-
Blood biochemistry				
Alanine aminotransferase, U/L	17.0 (15.0-22.0)	16.6 (13.8-33.7)	37.3 (18.2-45.0)	28.0 (21.0-29.5)
Aspartate aminotransferase, U/L	19.0 (17.0-24.0)	20.9 (17.4-26.3)	30.0 (20.4-39.1)	26.0 (22.5-27.0)
γ -glutamyl transferase, U/L	18.5 (17.8-20.5)	24.0 (14.0-44.0)	49.0 (24.0-62.0)	23.0 (19.0-38.5)
Total bilirubin, $\mu\text{mol/L}$	11.8 (9.4-14.6)	8.9 (5.2-11.3)	14.8 (8.4-19.8)	7.7 (5.2-11.4)
Direct bilirubin, $\mu\text{mol/L}$	1.4 (1.0-2.2)	4.5 (3.0-5.0)	5.4 (4.1-12.9)	2.1 (1.3-2.7)
Indirect bilirubin, $\mu\text{mol/L}$	7.2 (5.8-8.5)	4.3 (2.5-6.4)	6.3 (5.1-7.6)	-
Lactate dehydrogenase, U/L	-	208.0 (157.0-226.0)	340.0 (242.0-381.0)	139.0 (123.2-151.0)

C-reactive protein, mg/L	-	2.5 (1.2-7.6)	67.3 (14.2-121.6)	1.2 (0.8-8.2)
Procalcitonin, ng/ml	-	0.032 (0.031-0.043)	0.078 (0.051-0.154)	0.050 (0.050-0.070)
Creatinine, µmol/L	54.0 (43.0-59.0)	70.0 (63.0-74.0)	61.0 (50.0-64.0)	63.0 (53.0-82.0)
Urea, mmol/L	5.3 (4.4-6.1)	3.5 (3.2-4.4)	3.8 (2.8-5.2)	5.0 (3.6-5.4)
Uric acid, µmol/L	326.0 (264.0-373.0)	-	151.5 (106.0-232.5)	269.0 (225.5-389.5)
Glucose, mol/L	5.0 (4.9-5.5)	5.5 (4.9-5.8)	6.9 (5.4-11.8)	7.3 (5.5-8.3)
Total protein, g/L	72.0 (70.2-73.8)	67.1 (64.5-68.5)	62.6 (59.2-66.7)	70.3 (64.3-73.5)
Albumin, g/L	45.1 (43.3-46.5)	40.8 (38.8-41.6)	32.9 (31.4-36.7)	-
Globulin, g/L	27.0 (24.1-28.4)	26.0 (24.0-29.0)	30.0 (26.0-32.0)	26.3 (25.6-27.8)
Triglyceride, mmol/L	1.4 (1.1-2.0)	-	1.3 (0.9-2.0)	1.1 (1.0-1.4)
Total cholesterol, mmol/L	4.9 (4.5-5.9)	-	3.5 (3.0-3.8)	4.2 (3.8-4.4)
High density lipoprotein, mmol/L	1.3 (1.1-1.5)	-	0.9 (0.8-1.1)	1.2 (1.2-1.4)
Low density lipoprotein, mmol/L	3.0 (2.4-3.5)	-	2.2 (1.8-2.3)	2.2 (1.8-2.2)

^ano. (%): number.

^bSD: Standard Deviation.

^cIQR: Interquartile range.

Figures

Figure 1

Study design and metabolic profiling in serum samples from mild and severe COVID-19 patients a, Overview of cohort (including mild (n = 21) and severe (n = 23) COVID-19 patients and healthy controls (n = 17)) and the study design. b, t-SNE plot distributed healthy controls (n = 17), mild patients (n = 14) and

severe patients (n = 23) according to serum metabolites detected from targeted and untargeted metabolomics. c-d, Volcano plots comparing serum metabolites between mild (c) or severe (d) patients with healthy controls. Significantly increased and decreased metabolites are shown in red and blue, respectively, and metabolites with no statistical significance are shown in gray. Top 5 most significantly increased or decreased metabolites in each group are labeled. e, KEGG metabolic pathways in mild and severe patients enriched by significantly changed serum metabolites in c and d. f, Schematic depicting the key metabolic pathways altered in response to SARS-CoV-2 infection. Gray nodes represent metabolites that were not tested. Metabolite alteration are represented by color intensity, and borders are color-coded by statistical significance. Statistical significance was assessed using two-sided Mann-Whitney U test (c-d), and Fisher's exact test (e) followed by Benjamini-Hochberg multiple comparison test.

Figure 2

Metabolite-cytokine correlation in serum samples from COVID-19 patients a, Metabolic pathways enriched by metabolites that are significantly correlated with each presented cytokine. T statistics and statistical significance of metabolite-cytokine correlations were assessed based on two-sided t tests of regression coefficients followed by Benjamini-Hochberg multiple comparison test. Mean absolute (abs.) T statistics of metabolites in each pathway were calculated and represented by color intensity. Statistical

significance of pathway enrichment analysis was assessed by Fisher's exact test and followed by Benjamini-Hochberg multiple comparison test. b-e, Correlation networks of key CRS-related cytokines and metabolites significantly altered in severe patients. Nodes are color-coded by molecule types and metabolic pathways. Edges are color-coded by association directions. Networks were clustered by fast greedy modularity optimization algorithm. f-h, Chord diagrams of significant metabolite-cytokine correlations with metabolites involved in arginine metabolism (f), purine metabolism (g), tryptophan and NAD⁺ metabolism (h), respectively. Chords are color-coded by association directions consistent with b-e.

Figure 3

Longitudinal trajectories and metabolite-cytokine correlation in mild COVID-19 patients a, Longitudinal trajectory clustering of significantly changed serum metabolites, cytokines in follow-up patients with mild COVID-19. Metabolite and cytokine abundance in healthy controls were used as base line. Black lines represent the average trajectory for each cluster. b, Heatmap comparison of cytokines at distinct time point in follow-up patients. Log2 fold change of mean cytokine abundance in each interval relative to healthy controls is represented by color intensity. c, Enriched KEGG metabolic pathways in each cluster. d, Serum metabolite trajectories based on normalized data in follow-up patients. Blue solid lines pass through the mean of each measurement at the specific time interval, and dotted lines represent the mean of measurements in healthy controls. Generalized additive model (GAM) regression lines were represented by the black solid lines, with 95% confidence intervals for the regression line donated by gray filled areas. Data are presented as mean $\pm$ SEM. with individual data points shown. e, Chord diagrams of significant associations between metabolites and core CRS-related cytokines in cluster 1 (left), cluster 2 (middle), cluster 3 and 4 (right), respectively. Statistical significance of was assessed by Fisher's exact test (c) and linear regression model adjusted for age and gender (e) followed by Benjamini-Hochberg multiple comparison test.

Figure 4

Targeting metabolism modulates cytokine release in PBMCs ex vivo model a, Schematic representation of the experimental workflow. PBMCs, isolated from peripheral blood of mock-infected or SARS-CoV-2-infected rhesus macaques, were seeded in 96-well plates with vehicle or different drugs dissolved in the medium. 24 hours post seeding, cytokine abundance in cell culture was quantified. b-d, Metabolism diagrams and level of indicated cytokines and chemokines measured 24 h after supplementation of 1.25 mM arginine (b), 0.1 mM IDO1 inhibitor Epacadostat (c), and 0.1 mM IMPDH inhibitor Mycophenolic acid (MPA, d) in PBMCs. Data are presented as mean ± SEM. with individual data points shown. Statistical

significance was assessed using one-way ANOVA followed by Benjamini-Hochberg multiple comparison test.

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