Immunology of COVID-19

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Summary

The immune response to SARS-CoV-2 reveals a delicate balance between protective effects and harmful pathological reactions and can possibly explain the highly variable disease manifestations in subjects infected with this novel coronavirus. A better understanding of the anti-viral immune response is not only critical for vaccine development, but might also provide targets for pharmaceutical and immunological treatment options. Recent research literature on immune aspects of COVID-19 is summarized in this review with an outlook how bats have evolved to live with these viral infections.

Applied Immunology: On herd immunity and protection to reinfection

Herd immunity. A central concept in infection control is herd immunity, which is achieved when persons with protective immunity in a population have crossed a threshold preventing a further propagation of the pathogen even in the presence of non-immune subjects. The threshold for this transmission barrier depends on the "infectious force" of the pathogen, which is numerically expressed as the basic reproduction number R_0, i.e. the number of secondary infections caused by an index case. Herd immunity threshold and R_0 are linked by a simple mathematical function. SARS-CoV-2 has with an estimated R_0 of 2 to 3 a higher "infectious force" than influenza virus, but a lower than "flying infections" such as chickenpox or measles. A herd immunity of about 60% immune people is needed to stop the Covid-19 epidemic (Randolph and Barreiro, 2020). The initial strategy of the UK government was to let the epidemic roll over the country to achieve this herd immunity. However, this approach comes at a cost. You can calculate the cost of this strategy in human deaths from the infection fatality rate (IFR). In contrast to the case fatality rate (CFR) which gives the number of deaths per clinically ill patients (given with 1.4% to 15% for SARS-CoV-2 depending on region, time period and epidemiological setting), IFR is the number of deaths per infected subject. In industrialized

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countries the number of COVID-19-associated deaths is approximately known (despite some discrepancies between registered COVID-19 fatalities and excess mortality data at the peak of the Spring epidemic). The number of infected people is, however, not well known because many infections remain asymptomatic or mild and this number depends on the density of the testing program for viral RNA detection. A cumulative indicator of infected people is provided by the detection of serum antibody against SARS-CoV-2 as assessed in seroprevalence studies. The best current estimate for IFR is 0.6%; this is an average and varies strongly with age reaching up to 4% in older people. With IFR, one can calculate that achieving herd immunity by natural infection with SARS-CoV-2 without other protection measures will cost the life of 150'000 UK citizen. Frightened, the UK government changed strategy opting for a containment strategy. US epidemiologists showed in mathematic modelling that achieving herd immunity while simultaneously mitigating the impact of COVID-19 on hospital burden and fatalities is a challenging task. R_0 needs to be reduced from its initial value to about 1.2 to prevent a collapse of the health system. Subsequently, social distancing measures must be relaxed gradually in a highly controlled manner over a period of months to years. Even then success is not assured. A major unknown remains the nature, duration, and effectiveness of immunity acquired by natural infection (or later by vaccination) (Brett and Rohani, 2020).

Immunity passport. Many countries are now testing for SARS-CoV-2 antibodies at the population level or in specific groups, such as health workers. WHO supports these studies, as they are critical for understanding risk factors associated with infection. However, WHO warns that there is not enough evidence about the effectiveness of antibody-mediated immunity to guarantee an "immunity passport" for returning to high risk work places. People who assume that they are immune to a second infection because they have received a positive serological test result, may ignore public health advice and increase the risks of continued transmission (WHO 2020).

Recurrence. Concern about recurrence was initially raised by observing a return to a positive viral RNA test after 2 consecutive negative tests. Titers were, however, just around detection limit (Chen *et al.*, 2020b). After 2 negative tests in the hospital, a few persons from Wuhan tested positive for viral RNA 5 to 13 day after hospital discharge (Lan *et al.*, 2020). These cases are probably reflecting combined problems with false negative test results and prolonged virus excretion, frequently observed in COVID-19 patients. It is currently unclear to

what extent “long COVID-19” patients experiencing symptoms weeks and months after the initial infection are cases of recurrence **(**Rubin, 2020**).**

Reinfection. However, a few cases of true reinfection were documented. A 33-y old man from Hong Kong experienced a primary infection in March 2020 and suffered from cough, sore throat, fever and headache for 3 days. Saliva was positive by RT-PCR and yielded a virus sequence typical for a clade circulating in the US in Spring 2020, but he did not develop antiviral antibodies. He traveled to Spain and England in August and was tested again positive for viral RNA upon its return to Hong Kong. He did not show symptoms, but developed IgG antibodies against the virus. The second virus belonged to a clade circulating in England during Summer 2020 and differed from the first infection by 24 nucleotide changes scattered across the viral genome (To *et al.,* 2020). A 25-year-old male from Nevada without comorbidity or immune disorder developed sore throat, cough, headache, nausea, and diarrhea and tested positive for SARS-CoV-2 on April 18, the symptoms resolved and the man showed two negative tests in May followed at the end of May by another episode of fever, headache, dizziness, cough, nausea, and diarrhea, shortness of breath with a radiological diagnosis of atypical pneumonia. He tested positive for viral RNA on June 5, and showed serum IgM and IgG antibodies to the virus. Sequencing showed SARS-CoV-2 genomes in April and June that differed by 11 single nucleotide changes and one deletion and one insertion. Forensic tests excluded that the samples were derived from two different patients. (Tillett *et al.,* 2020) Reinfection with two different clades of SARS-CoV-2 viruses differing by 11 nucleotide changes was also reported for a Belgian 51 y-old women, who experienced headache, fever, myalgia, coughing, chest pain and dyspnea in March and after 3 months another infection with headache, cough and fatigue; she showed anti-viral antibodies. (van Elslande *et al.,* 2020) Symptomatic reinfection was observed in a 46-y old man from Ecuador who experienced intense headache and drowsiness in May after infection with a 20A clade virus, followed by a negative test in June, and developed in July severe symptoms including fever of 38.5°C, strong back pain, productive cough, and dyspnea after reinfection with a 19B clade virus, differing by 18 mutations from the first virus. The patient displayed IgM antibodies after the first and IgM and IgG antibodies against SARS-CoV-2 after the second infection. (Prado-Vivar *et al.,* 2020).

Some scientists concluded that reinfection cases tell us that we cannot rely on immunity acquired by natural infection to confer herd immunity (Iwasaki, 2020), but this conclusion is probably too pessimistic since it is currently based on only four cases of reinfection.

Reinfections with severe symptoms in the second episode as in the Nevada and the Ecuadorian patient are important for immunologists since such patients might show antibody-dependent enhancement of infection where antibodies bind to the virus, but do not neutralize the virus and even facilitate viral infection of immune cells, displaying receptors for the antibody. Such adverse immune reaction are rare, but have been observed during the vaccine development for the coronavirus causing MERS (Middle East Respiratory Syndrome). (Leford, 2020).

Indian researchers reported on two cases of silent reinfection in two health workers. In May both tested positive by RT-PCR without, however, showing any symptoms. Subsequently, both had a negative RT-PCR test. In August and September, respectively, these two health workers tested again positive for viral RNA in RT-PCR, again without showing any symptoms, but the PCR tests showed higher viral loads during the second infection. Both showed distinct viral strains differing by 9 and 10 unique mutations between the first and second episode. Seven variants mapped to predicted immune epitopes. (Gupta *et al.,* 2020)

Protection to experimental reinfections in primates. Chinese virologists infected rhesus macaques with 10^6 SARS-CoV-2 by trachea application. The animals showed a mild-to moderate disease course (weight loss, reduced appetite, radiological evidence of interstitial pneumonia, peak viral excretion 3 days after infection followed by gradual decline). Abundant $CD4^+$ T cells, $CD8^+$ T cells, B cells, macrophages, and plasma cells were found in the lungs, and serum anti-spike IgG antibody titers increased to 5000. The monkeys were re-challenged 28 days after primary infection with virus. No pathological change of the lungs were seen by radiology or histopathological sections, viral RNA was not detected, no immune cell invasion of the lungs was observed. As only reinfection sign the researchers observed a transient increase of temperature and a marked increase of anti-viral IgG serum antibodies titers to 80'000 and a tenfold increase of serum neutralizing antibodies to 320. (Deng *et al.,* 2020).

Humoral immune responses

Seroconversion. Understanding the immune reactions following natural infection or vaccination is crucial for predicting future trajectories of the pandemic and has led to intensive efforts by immunologists to decipher them. Three early Chinese studies investigated the antibody response to infection. In one study 285 Chinese COVID-19 patients were investigated for acute antibody response against the viral S and N proteins by a magnetic chemiluminescence enzyme immunoassay. Three weeks after symptom onset, 100% of the patients showed IgG antibodies. Of these patients, 63 patients were followed with multiple blood samples: 31% were IgG positive within 4 days after symptom onset. This prevalence increased to 69% after one week, and 90% after 2 weeks. IgM prevalence and titers were lower than those of IgG and IgM did not precede IgG antibody increases. Patients with severe disease had higher IgG antibodies than those with moderate disease. (Long *et al.,* 2020b). Then 112 patients from Wuhan with mild COVID-19 were investigated for serum antibodies by ELISA against the viral E and N proteins: 52% were positive for IgM and IgG, 41% were only positive for IgG. IgG titers were higher than IgM titers, and again IgM did not precede IgG antibody appearance. No antibody titer difference was seen in patients who became virus-negative compared to those that still excreted the virus (Zhang *et al.,* 2020b). Finally, 173 Covid-19 patients from Shenzhen were followed for IgM and IgG antibodies against the spike protein S in serial blood samples: 50% had seroconverted 2 weeks after symptom onset. IgM antibodies reached this prevalence 1 day earlier than IgG antibodies. After 3 weeks 90% of the patients had seroconverted. Critically ill patients had higher antibody titers than non-critical patients. Viral RNA titers did not significantly decrease with antibody appearance (Zhao *et al.,* 2020).

US researchers investigated convalescent plasma from 157 patients with proven COVID-19 infection 39 days after onset of symptoms. Overall, 78% and 70% of the plasma samples showed IgG antibodies against the Receptor Binding Domain (RBD) or the entire spike protein S, respectively. In contrast, only 15% and 34% of the plasma samples showed IgM responses to these viral antigens. Females showed lower titers than males. The overall level of neutralizing activity in the cohort was generally low, with 33% showing titers of less than 50. The geometric mean neutralizing antibody titer was 121, and only 2 individuals showed titers above 5,000. It is currently not known what neutralizing antibody titer is protective against

reinfection. Less than 0.07 % of all circulating B cells were directed against the viral antigens. (Robbiani *et al.,* 2020).

An international consortium investigated the antibody response in 47 hospitalized, 556 symptomatic and 44 asymptomatic SARS-CoV-2 infected subjects from Switzerland, Italy and the US. The IgG response was much higher in hospitalized compared to non-hospitalized individuals, higher in males than in females, but did not differ with age. RBD-specific reactivity dominated the IgG response, followed by antibodies against the viral nucleocapsid N. Anti-viral IgA was only found in hospitalized patients and virus-specific IgM was not limited to the acute stage of the infection. After antibody depletion with RBD-coated beads, neutralizing titers were reduced by 90%. They determined a half-life of 49 days for RBD-specific IgG and 75 days for S- and N-specific IgG, but observed an increase in antibodies blocking attachment to the cellular receptor ACE-2 for SARS-CoV-2 in half of the individuals, which they explained by affinity maturation. (Piccoli *et al.,* 2020)

Over 3 months after symptom onset, clinicians from London investigated the antiviral antibody response in 65 hospitalized patients with proven SARS-CoV-2 infection and in 31 infected health care workers. The cohort included the full breadth of COVID-19 severity. IgG and IgM responses against the spike protein S, the receptor binding domain RBD and the nucleocapsid N antigens were observed in more than 90% of the subjects, the IgA response was less frequent. A rapid decline in the IgM and IgA response was seen within 30-days after infection, while IgG response remained high up to 90 days. Peak neutralizing antibody titers were observed after 23 days when 8% had low (<200), 11% medium (<500), 19% high (<2000) and 60% potent (>2000) neutralizing titers. Disease severity enhanced the magnitude of the antibody response, but did not alter the kinetics. Comparison of the titers over time showed a decrease in almost all cases. (Seow *et al.,* 2020).

By investigating nearly 300 SARS-CoV-2 neutralizing antibodies from COVID-19 patients, US researchers found that the immunoglobulin G heavy-chain variable region 3-53 is used by 10% of patients. Notably, the germline-encoded residues dominate recognition of the ACE2–binding site, viral neutralization is thus achieved without much affinity maturation by somatic mutations. This variable gene region is also used by 0.5 to 2.6% of IgG of naïve uninfected humans. (Yuan *et al.,* 2020).

Monoclonal antibodies from COVID-19 patients defined six neutralizing epitopes on the SARS-CoV-2 spike protein, that differed in location, for accessibility in open or closed spike configurations, interaction with the ACE-2 receptor and cross-reactions with other coronaviruses. When using sera from COVID-19 patients in blocking assays against these six typing monoclonals, antibodies against two epitopes dominated the immune reaction: site Ia which largely overlaps the ACE2-binding site which is only accessible in the open S state; and site Ib which only partially overlaps with the ACE2 binding site and is accessible in both the open and closed states of the viral spike protein. (Piccoli *et al.,* 2020) US structural biologists investigated the binding modes of highly potent neutralizing antibodies (NAbs) and were able to distinguish different classes. Class 1 NAbs use the heavy chains encoded by the VH3-53 gene segment displaying a short 12 residues-long complementarity-determining region 3 that block ACE2 binding and could only bind the spike protein with RBD in the "up" position. These NAbs can crosslink adjacent RBDs within a single trimeric spike structure to achieve tighter binding. Class 2 NAbs use the same VH3-53 gene segment, but with a 15 residues-long complementarity-determining region. They overlap the ACE2 binding site and recognize both "up" and "down" RBD conformations of the viral spike protein. Class 3 NAbs bind outside the ACE2 binding site and recognize both "up" and "down" RBD conformations. (Barnes et al. 2020)

Antibody decay. Serum antibody responses to RBD were investigated by ELISA in 343 US American COVID-19 patients (93% required hospitalization, 53% intensive care and 13% died) over 122 days after symptom onset. Twenty days after symptom onset, 96% had specific IgG antibodies. The time to peak serum titers did not differ for the three isotypes IgG, IgM and IgA. Return to a seronegative status ("sero-reversion") was observed after 50 days for IgM and after 70 days for IgA, while only 3 patients became negative for IgG against RBD. These 3 patients had low IgG from the beginning and 1 patient was immunosuppressed. IgG antibodies to SARS-CoV-2 showed a slow titer decline. IgG titers demonstrated a good correlation with neutralizing titers and practically all patients showed a good neutralizing antibody titer 2 months after symptom onset. Cross-reactivity with seasonal coronaviruses was not observed. The authors found the relative persistence of IgG and neutralizing antibody responses encouraging, particularly since similar data were reported in a large survey in Iceland (Gudbjartsson et al., 2020), but noted that more rapid waning in anti-RBD titers was observed in studies analyzing antibodies in patients with mild or

asymptomatic SARS-CoV-2 infections. (Iyer *et al.,* 2020) Very similar serological observations were reported for 440 Canadian COVID-19 patients with modest decline in anti-spike and anti-RBD IgG, but rapid decline in IgM and IgA serum antibodies. Elevated anti-spike and anti-RBD IgG responses was also measured in saliva over 90 days. (Isho *et al.,* 2020) Over three months after symptom onset, US physicians reported a half-life of 36 days for IgG anti-RBD ELISA antibodies in 34 COVID-19 patients with mild disease. Greek physicians detected in plasma donors a significant antibody decrease over 2 months of follow-up (Ibarrondo *et al.,* 2020), which was also seen in patients from Japan (Yang and Ibarrondo, 2020).

Gender. US researchers studied 98 male and female COVID-19 patients matched for age. The patients were only moderately ill and did not receive drugs that could influence the immune response. Viral load and antibody response did not differ between the sexes. (Takahashi *et al.,* 2020) Differences between the sexes were subtle: Proinflammatory cytokines such as interleukin IL-8, IL-18, and the chemokine CCL5 were higher in male patients, while a more robust T cell response was observed among female patients. Analysis of their clinical trajectory revealed that clinical deterioration was associated with a poor T cell response in males and higher innate immune cytokine levels in females (Takahashi *et al.,* 2020). One study reported higher antibody titers in male than in female COVID-19 patients (Piccoli *et al.,* 2020).

Disease severity. As many subjects experience an asymptomatic infection, the quality of their anti-viral immune response is of interest. When screening 2,000 close contacts of COVID-19 patients under quarantine, 21% of those who became positive for viral RNA remained asymptomatic. Abnormal lung radiological findings were detected in 67% of them and a third of them showed elevated C-reactive protein levels, an infection parameter. Viral load was similar in asymptomatic and symptomatic patients, but the asymptomatic subjects remained virus positive in the nasopharynx for longer than the symptomatic patients. Both in the acute and the convalescent phase, antiviral IgG antibody levels were lower in 37 asymptomatic than in 37 symptomatic subjects. Both groups showed IgG and neutralizing antibody decreases but 13% of the symptomatic, in contrast to 40% of the asymptomatic subjects became seronegative in the convalescent phase. Asymptomatic individuals had a reduced inflammatory response compared to symptomatic patients when tested for 18 pro- and anti-inflammatory cytokines (Long *et al.,* 2020a).

Defective humoral immune response. Humoral responses in COVID-19 patients are often of limited durability and most antiviral antibodies exhibit limited somatic hypermutation. Long-lasting B cell memory and high affinity pathogen-specific antibodies mature within germinal centers in secondary lymphoid organs. US immunologists undertook an analysis in post mortem samples of thoracic lymph nodes and spleens from fatal COVID-19 infections. They examined lymphoid architecture and lymphocyte populations using multicolor immunofluorescence, multispectral imaging, and cell-cell interaction analyses. They observed the absence of germinal centers and a striking reduction in Bcl-6+ germinal center B cells. Absence of germinal centers correlated with an early specific block in Bcl-6+ T follicular helper cell differentiation. In parallel, abundant T helper 1 cells and aberrant tumor necrosis factor alpha (TNF-a) production were seen in COVID-19 lymph nodes. The loss of germinal centers led to the accumulation of non-germinal center-derived activated B cells. These findings also provide a mechanistic basis for the recent descriptions of non-durable humoral immune responses, impaired humoral immunity, and the low levels of somatic hypermutation in antibodies from convalescent COVID-19 patients. These data could explain the marked lymphopenia in COVID-19 patients, the absence of an IgM to IgG antibody class switching, the low serum neutralizing antibody titers which declined in some reports in patients already in a late convalescent phase. The robust activation of non-germinal center type B cell responses does not give rise to long-lived memory or high-affinity B cells. (Kaneko *et al.,* 2020).

Cellular immune responses

T cell response. German researchers investigated the cellular immune response in eighteen COVID-19 patients by isolating their peripheral blood mononuclear cells (PBMC) and stimulating them with peptide pools from the spike protein. Using flow cytometry they looked for activation markers identifying antigen-reactive CD4+ T cells. In 67% and 83% of COVID-19 patients they detected CD4+ T cells reacting against the N-terminal and the C-terminal spike peptide pool, respectively. Notably, most COVID-19 patients with critical disease exhibited no reactivity to the N-terminal peptides containing the RBD interacting with the ACE-2 receptor, suggesting a protective role of cellular immune response against the RBD-containing part of the spike protein. An unexpected finding was seen in healthy blood donors without exposure to SARS-CoV-2 as proven by negative viral RNA tests and negative antibody

tests against the SARS-CoV-2 RBD. In 68 negative controls, 35% had T cells that were activated - albeit at lower frequency-with the C-terminal spike peptide pool. They exhibited a memory phenotype and expressed interferon gamma (IFNγ), indicative of type 1 helper T cells (Th1) polarization similar to the T cells from COVID-19 patients. Since the C-terminus of the SARS-CoV-2 S protein shows a higher homology to human “common cold” coronaviruses (HCoVs), the researchers tested whether they detected cross-reacting T cells in their assay. This was indeed the case: T cells from control samples could be stimulated by peptides from seasonal coronaviruses. S-protein reactive CD4+ T cells from patients expressed CD38, HLA-DR and Ki-67, all markers characteristic for effector T cell responses during acute viral infections. T cells from healthy donors did not show these markers, reflecting an induction by infections that occurred in a more distant past. This observation has interesting implications. HCoVs account for approximately 20% of “common cold” upper respiratory tract infections and display a winter seasonality. Antibodies against HCoV wane within months after infection. Despite low or absent humoral immunity against HCoV, re-infection causes only low-level and short-lived virus shedding pointing to a protective role of the longer-lived cellular immunity, which apparently also shows cross-reactivity with the S protein from SARS-CoV-2. The authors speculate that cross-reacting T cells might explain the resilience of children against COVID-19 clinical symptoms, since children experienced more recent HCoV infections than older adults. (Braun *et al.,* 2020).

These observations were confirmed in cohorts from five different countries where 20 to 50% of people showed reactive T cells against SARS-CoV-2 without exposure to this virus. US immunologists investigated PBMC obtained before March 2018 in an *in vitro* stimulation assay against pools of 15-amino acid long peptides representing the entire translated SARS-CoV-2 genome. Overall, 54 % of positive responses occurred against the SARS-CoV-2 spike protein, but only 11% were directed against the receptor binding domain. Otherwise the reactive epitopes were evenly distributed over the remainder of the translated viral genome with the notable exception that no epitopes were detected over the viral membrane protein M. Staining tests identified the reactive T cells as effector memory CD4+ T cells. All reactive T cell were from subjects with serum antibodies against common cold-associated coronaviruses. In contrast, antibodies induced by seasonal coronaviruses did not cross-neutralize SARS-CoV-2. That the cellular reactivities were induced by exposure to these seasonal coronaviruses was shown by assays against genuine peptide epitopes derived from these viruses. The T cell

cross-reactivity was correlated with the degree of amino acid identity between the peptides of SARS-CoV-2 and the seasonal coronaviruses. Epitopes sharing more than 67% aa identity were likely to induce cross-reactive T cells. The authors speculate that this pre-existing cross-reacting cellular immune response against SARS-CoV-2 might explain part of the highly variable disease response towards infection with SARS-CoV-2 ranging from asymptomatic infection to severe life-threatening disease. The data are also important for vaccine development. The pre-existing T cell response does not induce an “antigenic sin” phenomenon, where an organism responds to the first encountered antigen when exposed to a new, but related antigen. Such a seasonal coronavirus boost was not seen in COVID-19 cases, reactivity was mainly directed against SARS-CoV-2 (Mateus *et al.,* 2020).

Swedish immunologists investigated in a systematic study T cell responses in the blood of acute and convalescent COVID-19 patients suffering from mild or severe disease symptoms; in exposed, but asymptomatic family members of COVID-19 patients; and in healthy blood donors sampled during and before the pandemic. Numbers of CD4+ and CD8+ T cells were unphysiologically low in COVID-19 patients. CD8+ T cells from acute COVID-19 patients expressed markers of activation and cell cycling, and excreted the cytotoxic compounds perforin and granzyme B. Length of time from exposure was associated with the emergence of stem-like memory SARS-CoV-2-specific CD8+ T cells. CD4+ and CD8+ T cells from convalescent patients expressed both interferon gamma (INFγ) and tumor necrosis factor (TNF). Viral spike protein-specific CD4+ T cells were skewed toward a circulating T follicular helper profile. Notably, cross-reactive T cell responses directed against either the spike or membrane proteins were detected in 28% of the healthy individuals, who donated blood before the pandemic; in 46% of blood donors during the pandemic; in 67% of exposed family members; in 87% of patients with mild and in 100% of those with severe disease. SARS-CoV-2-specific CD4+ and CD8+ T cell responses were present in 41% of seronegative individuals compared to 99% in seropositive subjects. Robust memory T cell responses were seen months after infection. Potent memory T cell responses were therefore even elicited in the absence of circulating antibodies, consistent with a non-redundant role of the humoral and cellular immunity against coronaviruses. (Sekine *et al.,* 2020) Whether this potent adaptive cellular immunity confers protection against primary infection and re-infection with SARS-CoV-2 needs to be determined in future studies.

Researchers from Singapore explored the T cell response by IFNγ ELISpot assays against structural proteins (nucleocapsid N, the most abundant protein in infected cells) and non-structural proteins from Orf1 (the earliest expressed proteins in infected cells) in 36 convalescent COVID-19 patients. Nearly all reacted against peptides of the N protein. Both CD4 and CD8 T cells were stimulated. Response against non-structural proteins was minimal. Next the researchers collected PBMCs from subjects infected with SARS in 2003. Notably, all subjects still showed a robust T cell response against the N protein, but not to the non-structural proteins of the SARS-CoV virus, indicating a long term persistence of memory T cells against coronavirus infection nearly two decades after infection. Interestingly, the T cells from former SARS patients also reacted with peptides from the N protein of the current SARS-CoV-2 strain, indicating a level of cross-reactivity not observed for neutralizing antibodies between the two coronaviruses. Then they extended the analysis to subjects without a history of exposure to SARS or COVID-19 as demonstrated by the absence of specific antibodies. These subjects also showed T cells that were stimulated by peptides from SARS-CoV-2. Interestingly, in unexposed subjects the T cell response was higher against the non-structural than against the structural proteins of SARS-CoV-2. Apparently memory T cells against coronaviruses persist for decades and are cross-reactive with SARS-CoV-2. (Le Bert *et al.,* 2020).

Disease severity. US immunologists measured SARS-CoV2-specific antibodies, and CD4+ and CD8+ T cells in 24 subjects with acute COVID-19 ranging from mild to fatal disease; in 15 convalescent subjects and in 15 unexposed controls to get an integrated view of the immune response against COVID-19. S (spike)- and N (nucleocapsid)-specific IgG and IgA (but not IgM) and neutralizing antibodies were seen in almost all acute and convalescent sera of patients. SARS-CoV-2-specific CD4+ T cells responding to the viral proteins S, M, N were detected in 77% of acute and 93% of convalescent cases, and consisted mainly of T follicular helper cells and IFNγ- producing cells, indicating antiviral polarization. SARS-CoV-2-specific CD8+ T cell responses expressing granzyme B were observed in 53% of acute and 87% of convalescent patients. In this cohort, CD4+ T cells, but not the presence of neutralizing antibodies were significantly associated with less severe disease. Specifically, one COVID-19 case had no detectable neutralizing antibodies, but resolved infection without hospitalization. In contrast, a fatal case showed an uncoordinated adaptive immune response, with neutralizing antibodies, but undetectable SARS-CoV-2-specific CD4+ T cell and CD8+ T cell responses.

To capture CD4+ T cells responding to SARS-CoV-2 in 40 patients with various severity of COVID-19 (18 non-hospitalized, 13 hospitalized, 9 intensive care patients), a UK-US consortium conducted in vitro stimulation of PBMCs with overlapping peptide pools targeting the immunogenic domains of the spike and membrane proteins of SARS-CoV-2. Then they isolated CD4+ memory T cells based on the expression of cell surface markers and analyzed single-cell transcriptomes of viral-reactive CD4+ T cells. CD4+ T cells are key orchestrators of anti-viral immune responses, either by enhancing the effector functions of other immune cell types like cytotoxic CD8+ T cells, natural killer (NK) cells and B cells or through direct killing of infected cells. They wanted to know whether different sets of CD4+ T cells are associated with different clinical outcome. In hospitalized compared to non-hospitalized patients, they found increased proportions of cytotoxic follicular helper (TFH) cells and cytotoxic T helper cells (CD4-CTLs) responding to SARS-CoV-2, and reduced proportion of SARS-CoV-2-reactive regulatory T cells (TREG). In hospitalized COVID-19 patients, a strong cytotoxic TFH response was observed early in the illness which correlated negatively with antibody levels to SARS-CoV-2 spike protein. Currently it is not yet clear whether the observed differences are the cause for the distinct clinical course or a consequence of the higher viral titers and interferon production in more severely affected patients. This study observed substantial heterogeneity in the immune cell composition between individual patients such that an analysis of 40 subjects does not yet allow generalizations. (Meckiff *et al.,* 2020)

Age. Adaptive immune responses were quite uncoordinated in patients older than 65 years-as compared to younger patients. Older patients showed dramatic losses in coordination between the CD4+ T cell and antibody responses. A small starting pool of naive CD8+ and CD4+ T cells was seen in older patients that may limit the development of a fast and large virus-specific T cell response, due to reduced starting material as a consequence of immune-senescence. (Moderbacher *et al.,* 2020) US physicians tested whether a distinct immune response is associated with the less severe infection as seen in pediatric cases. Young patients showed higher serum concentrations of interleukin IL-17A and IFNγ than adults. Adults mounted a more robust T cell response to the viral spike protein, and higher serum neutralizing antibody titers and antibody-dependent cellular phagocytosis than the pediatric cases. (Pierce *et al.,* 2020)

Gender differences. Differences between the sexes were subtle: Proinflammatory cytokines such as interleukins IL-8, IL-18, and the chemokine CCL5 were higher in male patients, while a more robust T cell response was observed among female patients. Analysis of their clinical trajectory revealed that deterioration was associated with a poor T cell response in males and higher innate immune cytokine levels in females (Takahashi *et al.,* 2020).

Immunopathology

Immune profiling. US researchers analyzed 125 COVID-19 patients using high dimensional cytometry, and integrated 200 immune and 50 clinical features in comparison to healthy individuals. A defining feature of hospitalized COVID-19 patients was the heterogeneity of their immune response. Overall, three immunotypes were defined: 1) patients with robust activation and proliferation of CD4 T cells, 2) patients with CD8 T cell responses, and less robust CD4 T cell and memory B cells responses, 3) and patients largely lacking detectable lymphocyte response to infection, suggesting a failure of immune activation in about 20% of the patients. The analysis established a link between group 1 which showed marked CD4 T cell activation and increased clinical severity score. Respiratory viral infections can cause pathology as a result of an immune response that is too weak allowing a virus-induced pathology, or an immune response that is too strong and leads to immunopathology. By localizing patients on an immune topology map, clinicians might in the future individualize therapeutic immunological interventions to specific patients. (Mathew *et al.,* 2020)

Systems biology analysis of immune response. US scientists used systems biological approaches (mass cytometry and single cell transcriptomics of leukocytes, transcriptomics of bulk PBMCs, and multiplex analysis of cytokines in plasma), to analyze immune response in 76 COVID-19 patients from Hong Kong or Atlanta compared to 69 controls. They observed an increase in the frequencies of plasmablasts (the precursor of a plasma cell) and effector CD8 T cells; effector T cells continued to increase up to day 40 after symptom onset, which might lead to exhaustion and apoptosis in T cells. Plasmacytoid dendritic cells (pDC) are bone-marrow derived antigen-presenting cells that secrete type-I IFN in response to viruses. In COVID-19 patients pDCs showed an impaired capacity to produce IFN-α and TNF-α in face of an early, transient type I IFN production in the lung tissue. They observed a paradoxically reduced expression of proinflammatory cytokines in myeloid cells (granulocytes and

monocytes), while plasma cytokines revealed enhanced levels. SARS-CoV-2 infection results in a spatial dichotomy in the innate immune response characterized by suppression of peripheral innate immunity in face of proinflammatory responses reported in the lung. Their data showed a temporal shift in the cytokine response from an early, but transient type 1 IFN response to a proinflammatory response during the later and more severe stages. There were enhanced levels of bacterial DNA and bacterial lipopolysaccharides in the plasma suggesting a role for bacterial products, perhaps of lung origin, in augmenting the production of inflammatory cytokines in severe COVID-19. These observations suggest a sepsis-like clinical condition (Arunachalam *et al.,* 2020).

***Viral sepsis hypothesis in severe COVID-19 patients*.** Only 5% of COVID-19 cases show severe infections leading in some to death due to severe lung injury and multiorgan dysfunction. Chinese clinicians observed that critically ill patients showed signs of shock with cold extremities, weak peripheral pulse, metabolic acidosis and microcirculation dysfunction. The symptom combination suggests septic shock, but in 76% of these patients SARS-CoV-2 is the sole pathogen. They proposed “viral sepsis” as cause for severe COVID-19 where the virus either infects the lymphocytes, or induces detrimental immune reactions and/or infects vascular epithelia leading to disseminated intravascular coagulation (Li *et al.,* 2020).

Clinical observations from patients in Wuhan concur with this concept. When investigating 48 COVID-19 patients with distinct disease severity, the researchers observed viral RNA in the blood (“RNAemia”) in 5 critically ill patients, 2 of whom died of respiratory failure. All 5 patients showed sharply increased interleukin IL-6 levels. Viral RNA in the blood and high IL-6 levels were biomarkers of severe disease (Chen *et al.,* 2020a).

The IL-6 link with severe disease was also observed by Greek researchers who compared immune activation and dysregulation in pneumonia patients caused by either bacteria, or influenza virus or SARS-CoV-2. At hospitalization COVID-19 patients were clinically less affected than patients with bacterial pneumonia. However, a common observation in COVID-19 patients was that they progressed from a relatively good clinical state into sudden deterioration one week after hospitalization. The researchers observed low expression of the human leukocyte antigen (HLA)-DR on CD14-monocytes in COVID-19 patients who needed mechanical ventilation. These patients showed a unique combination of defective antigen presentation and lymphopenia. IL-6 and C-reactive protein (CRP) were significantly increased

in severe cases. IL-6 is known to inhibit HLA-DR expression, and IL-6 showed indeed a negative correlation with HLA-DR in severe cases. (Giamarellos-Bourboulis *et al.,* 2020).

Cytokine storm hypothesis. The cytokine storm hypothesis of COVID-19 pathology is based on the pathology of acute respiratory distress syndrome (ARDS) where pneumonia, sepsis or aspiration pneumonia leads via a release of proinflammatory cytokines by immune cells ("cytokine storm") to severe lung damage. In COVID-19 the recruitment of the ACE-2 receptor by the virus causes disappearance of this angiotensin converting enzyme from the cell surface, which results in an increase of unprocessed angiotensin 2 (Ang II). Via a cascade of reactions, involving the metalloproteinase ADAM17, increased Ang II induces the cytokines TNFα and IL-6, which activate the IL-6 amplifier (IL-6 AMP), leading to the release of inflammatory cytokines. In parallel, the virus induces inflammatory cytokines by activating the NF-κB pathway via pattern recognition receptors (PRRs). This hypothesis explains a lot of the observed pathology in COVID-19 and offers many potential targets for pharmacological interventions. (Hirano and Murakami, 2020)

***Inflammation and thrombosis*.** US researchers demonstrated endothelial disruption and vascular thrombosis in histopathologic sections of lungs from both humans and rhesus macaques infected with SARS-CoV-2. They performed transcriptome analyses of bronchoalveolar lavage (BAL) and peripheral blood and serum proteome analysis in infected rhesus macaques. They observed macrophage infiltrates in lung and upregulation of macrophage, complement, platelet activation, thrombosis, and of proinflammatory markers, including C-reactive protein, MX1, IL-6, IL-1, IL-8, TNFα, and NF-κB. This upregulation of inflammatory and complement pathways leads to recruitment of macrophages and neutrophils, activation of platelets, and triggering of the coagulation cascade, explaining the microthrombi in the alveolar septa of SARS-CoV-2 infected animals and increased fibrin and blood clotting factors deposition in the lungs. The researchers described in the animals vascular intimal thickening with lymphocytic infiltrates, features of inflammation of blood endothelia that have also been observed in SARS-CoV-2 infected human. (Aid *et al.,* 2020).

Misfiring immune system. Immunologists from Yale University investigated the immune response in 113 COVID-19 patients over time in their blood samples. Some patients showed moderate disease while others developed severe disease. The longitudinal study design allowed a correlation between immune parameters and disease outcome. The analysis was

complex and limited to associations. However, a number of important observations can be gleaned from their analysis. Moderate and severe COVID-19 patients do not differ for a number of parameters. This is the case for CD4 T cells and CD8 T cells which are decreased compared to controls ("lymphopenia"). At baseline the nasal viral load does not differ between moderate and severe cases, but in moderate cases viruses declined over time which is not the case in severe cases. This observation fits with immunological correlates: at the beginning the researchers found increased levels of cytokines IL-1α, IL-1β, IFN-α, IL-17A in all patients – a reaction which they described as a 'core' COVID-19 immune signature. Severe disease was characterized by prolonged elevation of many cytokines, whereas the levels of most of them subsided in people with moderate disease in parallel with the decrease of the viral load. Apparently, there is a defect in the antiviral response in severe cases that does not achieve viral clearance and leads to a detrimental maintenance of an inflammatory response. The authors diagnosed a misfiring of the immune system. They discovered that parts of the immune system which is unrelated to viral control is triggered by the viral infection. Viral infection induce normally a type 1 immunity characterized by the expression of interferon-γ (IFNγ). Clearance of the intracellular pathogen is achieved by effector cells such a natural killer cells, cytotoxic T cells and T helper 1 cells. Paradoxically, SARS-CoV-2 infection also induces type 2 immunity, normally directed against helminth worm infections characterized by IL-4, IL-5, IL-13, and IgE responses and clearance by eosinophils and basophils. Even elements of a type 3 immunity against fungi was detected in COVID-19 patients with increased IL-17 levels. Unsupervised clustering revealed distinct profiles that influenced the evolution and severity of COVID-19. Cluster 1, characterized by low expression of proinflammatory cytokines and enrichment in tissue repair genes, leads to recovery. Clusters 2 and 3 were characterized by highly elevated proinflammatory cytokines, and led to worse disease or even death. (Lucas *et al.,* 2020; Perlman, 2020).

Interferons. IFNs are important cytokines of the innate and adaptive immune system and are classified into three main types: interferon I (α or β), II (γ), and III (λ). During viral infections, pattern recognition receptors detect viral nucleic acids, inducing the production of IFNs. IFN-γ for example binds and induces signaling through the IFN-γ receptor (IFNLR), which triggers an intracellular signaling pathway and induces a multitude of antiviral responses. Apparently, an early interferon response limits SARS-CoV-2 replication and assures a mild disease

process. If this mechanism does not work the body uses inflammation as defense, which, however, causes more harm than help, particularly in the lung.

French researchers investigated the interferon response in 50 COVID-19 patients with distinct disease severity compared to 18 healthy controls. Severe and critical disease courses in contrast to mild and moderate courses showed a markedly impaired type I interferon response with no detectable INF-β and low INF-α production. This compromised interferon production was associated with a persistent high blood viral load in the patients and an exacerbated inflammatory response. Inflammation was driven by the transcription factor NF-κB and led to a high TNF-α and IL-6 production. Type I interferons orchestrate a coordinated antiviral program. Low INF-α level preceded clinical deterioration and transfer to intensive care. The level of plasma INF-α was a characteristic biomarker for each disease grade in COVID-19 patients. Since the study had a cross-sectional design, it remained unclear whether reduced type I INF levels was present from the onset of disease (which could mean that SARS-CoV-2 has evolved strategies to shut down host INF production and can thus escape innate virus control), whether host genetic factors limit type I INF production or whether the interferon axis in severely affected patients was exhausted after an initial peak of interferon production. (Hadjadj *et al.,* 2020).

Immunogenetic aspects of COVID-19 pathology

Inborn errors in type I interferon genes. An international consortium of geneticists and clinicians investigated 650 COVID-19 patients with severe disease (14% of them died) and 530 patients with asymptomatic or benign COVID-19 infection by whole host genome or exome sequencing. Four unrelated patients with severe COVID-19 showed biallelic variants of the interferon regulatory factor 7 (*IRF7*) and of the interferon alpha receptor 1 (*IFNAR1*) gene. Further 119 subjects showed monoallelic variants in 12 loci including a number of immune regulatory genes such as Toll-like receptor 3 (*TLR3*), a pattern recognition receptors of the innate immune system; *IRF3, IRF7, IFNAR2, STAT 1* and *2* (transcription factors activated by interferons). These are all genes involved in type I interferon production and amplification pathways. Cells of these patients did not produce interferon I or III upon infection with SARS-CoV-2 or impaired IFNAR1 expression, phenotypes which could be

rescued by transduction of the corresponding wildtype genes. 10 of 23 patients showed very low interferon levels in the acute phase of the infection. (Zhang *et al.,* 2020a**)**

Autoantibodies against type I interferon. The same consortium investigated subsequently the presence of auto-antibodies against type I interferon since such auto-antibodies led to severe infections with other viruses. They found indeed IgG auto-antibodies against IFN-α2 and/or IFN-ω in 14% of 1000 patients with life-threatening COVID-19, but in none of 660 patients with mild or asymptomatic infections. The auto-antibodies were not induced by the infection because they existed already in the sera of some patients before the infection. The autoantibodies were biologically active because they prevented the activation of the interferon-induced transcription factor STAT1 and abolished protection otherwise mediated by added interferon in a cell culture infection test with SARS-Cov-2. The presence of auto-antibodies was associated with a poor outcome; death occurred in 37% of these patients. There was a striking excess of 94% males in patients showing critical COVID-19 pneumonia and neutralizing auto-autoantibodies against type I IFNs, suggesting an X-chromosome located risk gene for the generation of these auto-antibodies. (Bastard *et al.,* 2020)

Viral evasion from interferon control. Since the innate interferon (IFN) response constitutes one of the first lines of host defense against viral infections, viral evasion from interferon response is a common strategy of pathogenic viruses. US and Chinese virologists screened individual SARS-CoV-2 proteins for suppressors of IFN-I production and signaling. Three viral proteins (ORF6 and two non-structural proteins) blocked the antiviral response by interfering with the pathways leading from the detection of intracellular double-stranded RNA by a pathogen pattern detector (RIG-I) via the mitochondrial antiviral adaptor protein (MAVS) and two kinase activators to interferon regulatory factor 3, which induces interferon production. These 3 and two further viral proteins also interfered with the interferon signaling pathway triggering the expression of hundreds of IFN-stimulated genes (ISGs) with antiviral functions. (Xia *et al.,* 2020) Researchers from Hong Kong compared SARS-CoV and SARS-CoV-2, which both infect types I and II pneumocytes and alveolar macrophages. SARS-CoV-2 replicated in ex vivo human lung tissues more efficiently than SARS-CoV. SARS-CoV-2 failed to induce types I, II, or III interferons (INF), while SARS-CoV infection led to an increase in INF β, γ, and λ. As a consequence of this blunted INF response, SARS-CoV-2 infection only upregulated 38% proinflammatory cytokines/chemokines, while SARS-CoV

infection stimulated 85% of these key inflammatory mediators despite replicating less efficiently (Chu *et al*., 2020).

Further immunogenetic associations with severe COVID-19. A UK-US consortium expressed all 27 proteins encoded by SARS-CoV-2 (4 structural, 16 non-structural and 7 accessory proteins) individually in a cell line. The cells were then treated with UV to link the viral proteins to cellular RNA. From the lysed cells, they isolated the viral proteins and sequenced the associated RNA. Four viral proteins bound host RNA: Non-structural protein 16 (NSP16) binds to the mRNA recognition domains of the U1 and U2 RNA components of the spliceosome and acts to suppress global mRNA splicing. They observed a strong repression of interferon (IFN) responsive gene upon expression of NSP16. NSP1 binds to 18S ribosomal RNA region next to the mRNA entry channel into ribosome which leads to global inhibition of mRNA translation. Cells transfected with NSP1 and stimulated with interferon showed a strong repression of the IFN responsive gene transcription. Finally, NSP8 and NSP9 bind to discrete regions on the 7SL RNA component of the Signal Recognition Particle and thus interfere with protein trafficking to the cell membrane. Also co-expressed NSP8 and NSP9 led to a significant reduction in the IFN response. Since NSP1 would also paralyze viral protein synthesis, the 5' leader sequence added to each viral mRNA blocks NSP1 activity allowing viral mRNA translation. (Banerjee *et al.,* 2020)

In another genomic approach, 2000 COVID-19 patients from Italy and Spain hospitalized with respiratory failure were compared to 2000 healthy blood donors for a genome-wide association study evaluating 8 million single nucleotide polymorphisms. Two loci were associated with respiratory failure: (Ellinghaus *et al.,* 2020) Locus 3p21.31 on chromosome 3 comprised six genes (SLC6A20, LZTFL1, CCR9, FYCO1, CXCR6, and XCR1). The risk allele is associated with reduced expression of CXCR6 and increased expression of SLC6A20. CXCR6 regulates the specific location of lung resident memory CD8 T cells to airway pathogens, including influenza viruses. SLC6A20 encodes the proline transporter SIT1, which functionally interacts with angiotensin-converting enzyme 2, the SARS-CoV-2 cell-surface receptor. LZTFL1 gene (leucine zipper transcription factor) is involved in organelle biogenesis of ciliary membranes (suggesting a role in mucus transport?). Possession of this haplotype conferred a 1.6 higher risk for experiencing severe COVID-19 requiring hospitalization. The haplotype occurs in South Asia at a frequency of 30% with a

focus in Bangladesh where 63% carry at least one copy of the risk haplotype and 13% of the population is homozygous for the haplotype. This concurs with the epidemiological observation that India and Bangladesh are hotspots of the COVID-19 epidemic and that individuals of Bangladeshi origin in the UK have an about two times higher risk to die from COVID-19 than the general UK population. (Zeberg and Pääbo, 2020)

A second association was found on chromosome 9, locus 9q34.2, and implicated the involvement of ABO blood groups in COVID-19 susceptibility. Subjects with blood group A were at higher (odds ratio: 1.45) and those with blood group O at lower (odds ratio: 0.65) risk of developing severe COVID-19 disease. The biologic mechanisms for an association with blood groups might be that it influences the development of neutralizing antibodies against protein-linked N-glycans (Ellinghaus *et al.,* 2020).

A new study including many intensive care COVID-19 patients could identify further risk genes: IFNAR2 that encodes a cell receptor for interferon, DPP9 known to be involved in lung disease and TYK2 that encodes a signaling protein involved in inflammation. There are existing drugs for the last two gene functions used in diabetes and arthritis patients (Kaiser, 2020).

Finally, exome sequencing from two young adult brothers both requiring mechanical ventilation, one of them died, revealed loss-of-function variants of the X-chromosomal gene *TLR7,* the Toll-like receptor 7 which plays an important role in pathogen recognition and activation of innate immunity. Type I interferon (IFN) signaling was transcriptionally downregulated in their mononuclear blood cells. The production of IFN-γ was decreased when stimulated. (van der Made *et al.,* 2020)

How do bats cope with coronaviruses?

Bats are efficient transmitters of zoonotic infections. Their species richness, mobility, longevity and lifestyle (dense colony dwellers) favor viral infections. However, bats found a solution to deal with viruses since experimental infections even with highly lethal viruses do not cause disease in bats. It is therefore of high interest to study how bats deal with viral infections (Gorbunova *et al.,* 2020). Bats have a robust interferon response to RNA viruses: they constitutively express IFN-a, but counteract inflammation by a dampened activation of the

NLRP3 inflammasome and by downregulating TNF-a expression. Naked DNA in the cytoplasm is a sign of viral infection (or of damaged mitochondria) leading to chronic inflammation and senescence ("inflammaging"). Bats lack PYHIN genes, which activate inflammasomes and lack genes that drive type I IFN gene transcription such as IFI16. Upon binding to cytosolic DNA as alert signal, cyclic GMP-AMP synthase binds and activates STING which triggers the type I IFN response. Also this pathway is dampened in bats. The Toll-like receptor TLR9 in bats shows reduced activation by CpG oligodeoxynucleotides. Proinflammatory response in macrophages from bats are associated with expression of the anti-inflammatory cytokine interleukin IL-10. These immunological observations seem at first glance counterintuitive since they dampen the immune response rather than activating it. Apparently, we can learn from bats that to co-exist with viruses, controlling inflammation might be more important than ramping up the immune system to combat the virus.

According to some immunologists, bats found after 60 million years of evolution a solution for allowing a lifestyle which exposes them to massive viral exposure. Humans have only recently been exposed to crowded and mobile lifestyles and selection has not yet had enough time to prepare us for the dangers associated with this new lifestyle. It is not only the human crowding in mega-cities that increases the risk of viral exposure. To feed soon 10 billion humans on a not expanding earth causes humans to transform more and more natural ecosystems for food and feed production. These environmental encroachments bring humans close to wildlife leading to increased exposure to their viruses and increased zoonosis risks. Mobile animals such as bats in turn invade our agricultural and urban environments in search for novel food sources adding to the zoonotic pressure (Hendravirus, Nipahvirus) (Brüssow, 2012). As bacteria defend their niche with bacterial viruses and phage-derived bacteriocins, animals might use the same strategy in using animal viruses adapted and hence harmless to them, but virulent to other species. The sentinel infectivity test of placing a monkey in a cage at the entrance of a bat cage before entering it is a lively illustration of this principle. Comparing prokaryotic viral defense systems with those of animals is not so farfetched as recently demonstrated by the origin of the stimulator of interferon genes (STING) as an antiviral system shared by prokaryotes and mammals (Morehouse *et al*., 2020). It might therefore not be exaggerated to invoke another hypothesis in prokaryotic viral ecology. In marine microbial ecology there is a popular "killing the winning" hypothesis where viruses serve to maintain species diversity against overgrowth by a single species (Wommack and Colwell, 2000).

Transferred to terrestrial ecosystems, we humans are the “winning” population and are now likely to feel the pressure of viruses from animals, for host restriction reasons mostly from mammals and birds, which we are displacing. With environmental and climate changes, these zoonotic viral pressures are likely to increase. Scientific research must provide insights and solutions how to cope with these problems which evolution cannot provide due to the quick speed with which we changed our environment. (Gorbunova *et al.,* 2020).

To explore this potential, an international consortium has sequenced the genomes of six bat species to a depth only surpassed by mice and men. They detected positive selection on several immunity-related genes which include interleukins involved in immune regulation, activation of transcription factor NF-κB and proteins involved in responses to pathogens that may have contributed to the unique tolerance of viral pathogens among bats. Bats evolved immunomodulatory mechanisms that enabled a higher tolerance to pathogens than is typical amongst mammals. The researchers identified immune gene losses that potentiate cellular responses to multiple cytokines and amplify NF-κB activation mediated by bacterial lipopolysaccharides. In addition, they identified an expansion of the APOBEC (apolipoprotein B mRNA editing catalytic polypeptide-like) which is a family of evolutionarily conserved cytidine deaminases displaying anti-viral functions. These insights might identify new druggable targets for pharmacological interventions in humans against SARS-CoV-2 and future emerging viruses (Jebb *et al.,* 2020).

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