*Ciencia, Tecnología y Salud*
Vol. 7 Num. 3 2020
ISSN: 2410-6356 (electrónico) / 2409-3459 (impreso)

**Artículo de Revisión / Review Article**

# NK cells at the crossroads in COVID-19: a question of timing

*Células NK en la encrucijada en COVID-19: una cuestión de tiempo*

Susana del Toro-Arreola[1,2], Fabiola Solorzano-Ibarra[1], Martha C. Téllez-Bañuelos[3], Jesse Haramati[3]*

[1]Instituto de Enfermedades Crónico Degenerativas, Departamento de Biología Molecular y Genómica,
[2]Laboratorio de Inmunología, Departamento de Fisiología, Centro Universitario de Ciencias de la Salud,
[3]Laboratorio de Inmunobiología, Departamento de Biología Celular y Molecular, Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Guadalajara, México

***Autor al que se dirige la correspondencia: jharamati@gmail.com**

Recibido: 25 de julio 2020 / Revisión: 25 de agosto 2020 / Aceptado: 08 de septiembre 2020

## Abstract

The outbreak of the novel coronavirus SARS-CoV-2 and the attendant physiological symptoms associated with the COVID-19 disease have led to an explosion of interest studying different aspects of the immune response. As of yet, the particular roles of natural killer cells are not well understood in this disease. NK cells are critical first-response cytotoxic cells of the innate immune system. NK cells are traditionally considered important for their roles in innate immunity against tumors and viral infected cells, as well as their ability to produce cytokines, particularly interferon-γ, and participate in antibody dependent cell cytotoxicity (ADCC). Here, we describe the role of NK cells in peripheral blood and in the lungs with respect to the pathology caused by SARS-CoV-2 and discuss the implications of proposed different types of therapies on NK cells. Evidence is accumulating that NK cells play an important role in initial surveillance as part of innate immunity. With the progression of the disease and rising inflammation, these cells, when in circulation, appear to become exhausted and ineffective. In the COVID lung, however, a complex interplay between inflammatory cells, chemokines, cytokines and aberrantly activated migratory NK cells occurs, potentiating local inflammation and the critical situation in the lungs.

Keywords: NK cells, COVID-19, Immune response, Innate Immunity, SARS-Cov-2

## Resumen

El brote del nuevo coronavirus SARS-CoV-2 y los síntomas fisiológicos concomitantes asociados con la enfermedad COVID-19 han provocado una explosión de interés en la investigación de diferentes aspectos de la respuesta inmune. Hasta el momento, no se comprenden bien las funciones particulares de las células asesinas naturales (NK, por sus siglas en inglés: *natural killer*) en esta enfermedad. Las células NK son importantes células citotóxicas de primera línea que forman parte del sistema inmune innato. Las células NK se consideran tradicionalmente importantes por su papel en la inmunidad innata contra tumores y contra células infectadas por virus, así como por su capacidad para producir citoquinas y participar en la citotoxicidad celular dependiente de anticuerpos (ADCC, por sus siglas en inglés: *antibody-dependent cell-mediated cytotoxicity*). Aquí, se describe el papel de las células NK en sangre periférica y en pulmones con respecto a la nueva patología causada por SARS-CoV-2 y discute las implicaciones de los diferentes tipos de terapias propuestos con respecto a células NK. Al momento, diversos tipos de evidencia comienzan a revelar que las células NK podrían desempeñar un papel crucial en la vigilancia inicial contra el SARS-CoV-2. Con la progresión de la enfermedad y el aumento de la inflamación, estas células cuando están en circulación, parecen agotarse (*"exhausted"*) y volverse ineficaces. En los pulmones de pacientes con COVID-19, sin embargo, se produce una interacción compleja entre células inflamatorias, quimioquinas, citoquinas y células NK migratorias activadas de manera aberrante, lo que potencia la inflamación local, contribuyendo a una situación más crítica a la función pulmonar.

Palabras claves: Células NK, COVID-19, Respuesta inmune, Inmunidad innata, SARS-CoV-2

## Introduction

The novel coronavirus (2019-nCoV; Severe Acute Respiratory Syndrome Coronavirus 2 -SARS-CoV-2-) emerged in December 2019 in Wuhan, China. Rapid global transmission focused world-wide efforts on understanding and controlling the emerging pandemic. While the world first focused on understanding its symptoms and diagnosis, it was soon apparent that the host response to this beta coronavirus, with 79% similarity to the virus in the 2003 SARS outbreak, and about 50% similarity to the virus in the more deadly 2012 MERS (Middle East Respiratory Syndrome) outbreak (Lu et al., 2020), yet not so far removed from the viruses that cause some common colds, was exceedingly complicated.

As a virus that in humans causes a respiratory syndrome, SARS-CoV-2 infects type I and type II alveolar epithelial cells of the lungs, as well as alveolar macrophages that are among the first producers of pro-inflammatory cytokines. COVID-19 is characterized by progressing stages: a pre- or a symptomatic incubation stage, with increasing viral load and contagiousness; the first symptomatic stage, usually 5-11 days post infection and characterized by dry cough, headache, fever, shortness of breath, fatigue, diarrhea, and/or loss of taste or smell; after about a week post the onset of the first symptoms, the next stage manifests with dyspnea and viral pneumonia that involves the lower respiratory tract, and inflammatory markers rise. A minor proportion of patients experience hypercytokinemia (the often-named cytokine storm) and, with systemic inflammation increasing, progress to the rapid onset of acute respiratory distress syndrome (ARDS) and multi-organ failure (Oberfeld et al., 2020).

This inflammatory state is characterized by high levels of C-reactive protein, D-dimer, and a broad array of cytokines such as IL-6, IL-1β, TNF-α, and IL-8, and the infiltration of inflammatory and degranulating cells, such as monocytes, monocyte-derived inflammatory macrophages, neutrophils, and NK cells, into the lungs (Liao et al., 2020; Merad & Martin, 2020). The time from the onset of symptoms to the development of ARDS ranges from 8 to 12 days, and the median time to intensive care unit (ICU) admission from the onset of illness or symptoms ranges from 10 to 12 days. Symptoms and disease progression vary widely between patients of different ages and with different comorbidities (Centers for Disease Control and Prevention, 2020). To give a general overview, one of the first of the large population studies (out of China) found: mild to moderate (mild symptoms up to mild pneumonia) in 81% of patients; severe (dyspnea, hypoxia or > 50% lung involvement on imaging) in 14% of patients; and critical (respiratory failure, shock, or multiorgan system dysfunction) in 5% of patients (To et al., 2020).

With such a significant difference in physiological responses to the same viral infection, interest naturally turns to questioning what other variables might be important. Increased testing in many countries has revealed that higher percentages (than reported at the beginning of the pandemic) of infected persons might be asymptomatic or paucisymptomatic; that is, capable, in some unknown way, of eliminating the virus before any, or the more severe, pathological effects are manifested (He et al., 2020; Li et al., 2020; Oran & Topol, 2020). Great interest has focused on the adaptive immune system, principally the production of neutralizing antibodies, for the control of this disease. However, the production of new antibodies lags viral infections, and typically takes about 7-10 days. How then, to explain the difference in immune response, which, in some cases, appears to initiate from the first moments of viral infection?

As sentinel antiviral cells of the innate immune system and potent producers of cytokines, this raises the question, are NK cells important in COVID-19? NK cells secrete proinflammatory cytokines which inhibit viral replication, stimulate the adaptive immune response and recruit other immune cells. On the other hand, the hyperactivity of these cells might contribute to the symptoms observed in the severest COVID-19 cases.

## Content

### NK cells in SARS-Cov-2 infections and COVID pathologies

#### NK cell definition

NK cells are the major cytotoxic effector cells of the innate immune system. In contrast with the adaptive immune system, innate immunity is constitutively active and broadly reactive to a plethora of danger- or pathogen-associated molecular patterns. The NK cell component of this system is comprised of about 2 to 18% of the lymphocytes in peripheral blood (Vivier et al., 2008). NK cells are large granular cells, now known

as members of the innate lymphoid cell (ILC) family, that are primarily characterized by their ability to kill target cells. (Stokic-Trtica et al., 2020). This cytotoxic activity is regulated by the "missing self" postulate (Ljunggren & Kärre, 1990). Due to the extreme power and reactivity of NK cells, their activation is tightly regulated by an interplay between two classes of receptors: activating (generally specific for stress-related, tumor or viral proteins) and inhibitory (generally self MHC molecules). The lack of an inhibitory signal allows NK cell activation to proceed. The term "missing self" describes target cells that do not have self MHC class I expression. Downregulation of MHC class I is a mechanism to evade immune responses by cytotoxic CD8 T cells, often found in tumors and viral infections. This explains why NK cells are often critical in the immune response to tumors and viruses. Additionally, NK cells are activated by the induced-self model, that is, the up-regulation of danger signals or stress-related proteins in tumor or virus infected cells (Diefenbach & Raulet, 2001).

Human NK cells are traditionally classified into two subsets(Lanier et al., 1986): the vast majority are $CD56^{dim}$ cells, which are cytotoxic and also strongly express CD16, the receptor for the common Fc region of IgG (FcgRIII); thus participating strongly in ADCC (antibody-dependent cell cytotoxicity), the process by which NK cells induce apoptosis in target cells that are covered with antibodies. A minority of NK cells are $CD56^{bright}$ cells, these $CD16^{dim}$ or negative cells, are usually considered immature with respect to their $CD56^{dim}$ counterparts, and are biased toward cytokine production, and additionally may either have immunoregulatory properties, or become cytotoxic in different models and situations (Michel et al., 2016). Apart from these two divisions, it is important to note that the rapidly expanding world of NK cell subpopulations can be divided by maturity status (CD57 and KIR positivity marks terminal differentiation and NKG2C+ marks antigen experienced mature cells; CD62L marks circulating versus tissue resident $CD56^{bright}$ NK cells) and activation state (presence of CD107a, perforin, granzyme B), as well as the presence of cytokine receptors and, more recently explored, checkpoint molecules (Abel et al., 2018; Michel et al., 2016).

In addition to the above, the lines between NK cells and other cells of the innate and adaptive immune system have been blurring in recent years. NK cells, besides utilizing the familiar cytotoxic T-cell mechanisms of Fas ligand, perforin and granzyme B, and TNF/TRAIL in inducing apoptosis in their targets, are also now recognized to participate in cross talk with various immune cells, sense pathogens using TLRs and other pathogen associated pattern receptors to surveil local environments (Vitale et al., 2019), and, in contrast with earlier views of these cells, exhibit a limited degree of immunological memory (Pahl et al., 2018). This last point is intriguing, but, due to the lack of reports of pre-existing populations of such cells within patient groups with respect to COVID-19 is outside the scope of this review. However, memory-like NK subsets may explain differing immune responses to SARS-CoV-2 in diverse populations, most notably children, that might have previously experienced high exposure to other viruses; the investigation of such NK cells represents another important topic for future studies with respect to COVID-19.

## NK cells in the blood

Aside from systemic inflammation, one of the most salient observations regarding COVID-19 is the onset of systemic lymphopenia, neutrophilia, and extreme neutrophil to lymphocyte ratios (Arentz et al., 2020; Bhatraju et al., 2020; Giamarellos-Bourboulis et al., 2020; Wang et al., 2020). Patients who died from COVID-19 were found to have had markedly lower lymphocyte levels than survivors. Currently, many (though not all) groups have reported a decrease in NK cells in the blood, along with the well reported T cell decrease. In one of the first of these studies, this decrease was more marked in severe versus mild disease sufferers, and was also accompanied by increased IL-2, IL-6, IL-10 and TNF-α (Tan et al., 2020). Another study associated loss of NK cells in the blood with progression of the disease, age and age-related comorbidities, such as dyslipidemia, diabetes or hypertension (Jurado et al., 2020).

A different study across a heterologous group of COVID-19 patients found only a moderate association of decreases in NK cells with disease severity, while finding hyperactivation of a small group of CD4 and CD8 cells in the G1 phase of the cell cycle, yet an overall depletion of T cells (most markedly in CD8, CD4 [Th1, Th17] and gamma delta T cell populations), and depletion of basophils and plasmacytoid dendritic cells (Laing et al., 2020). Again, a strong association with pro-inflammatory cytokines was seen, with a notable upregulation of CXCL10 (also known as IP-10). CXCL10, perhaps produced by infected airway

cells (Spurrell et al., 2005) or activated neutrophils, is a chemoattractant and activator of many mononuclear cells, notably NK cells (Tokunaga et al., 2018), and has been seen increased in other viral airway pathologies (Zeng et al., 2005). Notably, this immunophenotype was found to be similar to that observed in MERS, SARS-CoV-1, and sepsis (Laing et al., 2020).

While a drop in total lymphocyte counts has been widely reported, some groups have found no or minimal change in the blood of total NK cell levels (Varchetta et al., 2020).

It is important to note that these observations were found in patients with minimal disease symptoms. Another group found a biphasic response when evaluating mild versus severe cases. In this report, they found increased blood NK cells in mild versus both healthy donors and severe or critical patients. The increased NK activity in the mild patients was accompanied by increased IFN-γ and decreased IL-6 and IL-10, while the severe patients were characterized by significant loss of NK cells, and inhibition of the IL-2 pathway in CD8 cells, thus highlighting the different immune responses between mild and critical cases, and implying that NK activation may be part of the effective antiviral immune response in some cases (Shi et al., 2020).

Finally, different NK subpopulations have been described in the blood. When looking at different NK subpopulations it becomes more difficult to see a clear pattern of inhibition or expansion with regards to the progress of the disease. Both the majority $CD56^{dim}$, and the rarer $CD56^{bright}$ NK cells were found to be decreased in patient peripheral blood. This study found that the rare subset of adaptive NK cells, which are $NKG2C^{+}$ $CD57^{+}$, were increased in severe cases, as were $CD56^{bright}$ cells with a cytotoxic profile (perforin, Ksp37, MIP-1β, CD98, Tim-3 and granzyme B positive) (Maucourant et al., 2020). Another study found the related CD57+/FcεRIγ- adaptive NK cells to be increased in patients and associated with poor prognosis as well (Varchetta et al., 2020). One report found that activated CD56- CD16+ NK cells, highly cytotoxic cells, which, along with $CD56^{dim}$ NKs, mediate ADCC, were increased (Marcos-Jimenez et al., 2020).

Using single-cell RNA-sequencing on peripheral blood from healthy donors and patients, a different group found decreased NK cells (both $CD56^{dim}$ and $CD45^{bright}$) and decreased frequencies of myeloid cells, including plasmacytoid DCs and CD16+ monocytes. Transcripts from patient NK cells showed increased Lag-3 and Tim-3 (checkpoint markers) and activation marker transcripts, and decreased maturation and cytotoxicity transcripts (CD16, Ksp-37 and granulysin) in the severest patients (Wilk et al., 2020). Similarly, another recent report found a sharp decrease in total NK cells and mature CD16+ CD57+ NK cells in the blood and lungs of the sickest patients with ARDS, while an increase in cells with an immature CD57- phenotype, along with an increase in checkpoint molecules, which will be discussed below (Demaria et al., 2020).

## Exhausted NK cells in the blood

In addition to the loss of NK cells in the blood, and changing NK populations, a critical question turns on the fitness of the NK cells. It is important to highlight that this fitness is not only with respect to the traditional cytotoxic NK role in the elimination of virally infected cells, but that NK cells also dampen local inflammatory responses by eliminating inflammatory cytokine producing dendritic cells, monocytes and T cells (Malhotra & Shanker, 2011).

In COVID-19 patients, increased production of inflammatory cytokines inversely correlated with perforin-expressing NK and T cells, with these potentially cytotoxic cells greatly decreased in ICU versus non-ICU patients (Bordoni et al., 2020). While at first glance this observation may appear an association without causation, the over production of IL-6 has been shown to be inhibitory to NK cells and linked to decreased perforin and granzyme mediated cytotoxicity (Cifaldi et al., 2015). In a related study with COVID, similar associations were observed and the cytotoxic activity of the NK cells was restored using the anti-IL-6R monoclonal antibody tocilizumab (Mazzoni et al., 2020).

Peripheral blood T and NK cells have been found to express the checkpoint molecule PD-1 in a number of studies (Diao et al., 2020; Varchetta et al., 2020; H. Zheng et al., 2020). In adaptive NK cells, which -as mentioned in one study above- were one of the few lymphocyte populations to increase with disease severity, PD-1 was found to be increased (Maucourant et al., 2020). PD-1 is more commonly investigated on T cells, but it has been shown that a subset of NK cells can express PD-1 and that these cells had a mature phenotype $KIR+CD57+CD56^{dim}$ and had been over stimulated via NKG2A (Pesce et al., 2017). In light of this, the finding that NK cells may over express the inhibitory molecule NKG2A in severe COVID-19 cases is particularly notable (Demaria et al., 2020;

M. Zheng et al., 2020). The Zheng group additionally found expression of NKG2A to increase, while the percentages of T and NK cells expressing the activation marker CD107a and producing IFN-ɣ, IL-2, and TNF-α significantly decreased in patients. In recovered patients, peripheral NK cell numbers rebounded and, correspondingly, NKG2A was found to decrease upon recovery (M. Zheng et al., 2020). A more recent work also found increased PD-1 percentages and NKG2A cell surface expression on NK cells (from both the blood and lungs) from patients with ARDS. In a further experiment, this report examined these patient peripheral blood NK cells for their ability to lyse model target tumor cells. This group convincingly found that anti-NKG2A blocking antibodies could be used to inhibit the NKG2A-HLA interaction on these cells, and that the blocking antibody treated cells regained their cytotoxic ability, thus providing critical evidence for the use of anti-NKG2A therapies as a possible tool for hyperactivating NK cells in COVID-19 (Demaria et al., 2020). While PD-1 was not evaluated in these studies with NKG2A, it would be very interesting to see if PD-1 likewise decreased in the recovered patients in these groups, and if anti-PD-1 blocking monoclonal antibody treatment could rescue cytotoxic activity in patient NK and T cells.

In line with the above, IL-6, IL-8 and IL-10, reported in various studies to have been significantly augmented in patients with COVID-19, have also been reported to inhibit NK cells and to increase NKG2A expression in NK and naive CD8+ T cells (Wu et al., 2019; Wu et al., 2017). Moreover, IL-6, which inhibits NK cells in a STAT3 dependent mechanism (Wu et al., 2019), may further impair NK activity by reducing the expression of NKG2D, an activating receptor for NK cells (Osman et al., 2020). NKG2D expression may also be inhibited by soluble stress-induced molecules MICA/B (Arreygue-Garcia et al., 2008), these NKG2D ligands are liberated in some tumors and some viral infections, though they have yet to be described in the context of COVID-19. Other groups have found a decrease in NKp30, NKp46, and NKG2D in deceased versus surviving patients (Varchetta et al., 2020).

In contrast to the above studies, one report has observed the hyperactivation of relatively rare $CD56^{bright}$ cells to be correlated with the severest disease. Here, increased perforin and granzyme in these cells correlated negatively with the checkpoint molecule TIGIT, suggesting that using checkpoint agonists might be a possible therapy to inhibit overactive subsets of NK cells in advanced disease (Maucourant et al., 2020). ADCC is theorized to be a potential role of these cells (traditionally $CD56^{dim}$ NK cells are considered mediators of ADCC); however, it remains to be seen how relevant ADCC is in severe illness, or if it is even protective or pathogenic. An anti-SARS-CoV-2 neutralizing antibody, s309, has been shown to induce ADCC, but it is not clear if this would occur with the same kinetics in severe illness (Pinto et al., 2020).

The role of age in the current pandemic has received great attention. The elderly (generally speaking people aged 60 and above), even when mostly healthy, exhibit greater numbers of exhausted T and NK cells, and chronic low-grade sterile inflammation, characterized by high serum concentrations of C reactive protein, IL-6 and IL-8 (Akbar & Gilroy, 2020). Additionally, senescent cells, which potentiate the pro-inflammatory milieu, increase in the elderly, and are associated with impaired lung function (Campisi, 2016). Similarly, it has been shown that perforin expression by NK cells declines significantly after the age of 70 years and that children have higher levels than adults (Cunningham et al., 2020).

This leads to an interesting proposal by Cunningham et al. (2020), that the pathology of severe COVID-19 cases might be similar to other diseases where deficiencies in perforin and cytotoxic cells exist. Examples of these diseases are macrophage activation syndrome and hemophagocytic lymphohistiocytosis. The above authors make the case that an overactive inflammatory state and cytokine storm are hallmarks of such syndromes. Sex differences are also reported, with adult women having consistently higher levels of perforin than do men of equivalent age, a clue, perhaps, that might help explain the greater susceptibility in males to severe forms of the disease. As of yet, deficiencies in perforin pathways have not been found in the rare SARS-CoV-2 related disease, Multisystem Inflammatory Syndrome in Children, however this Kawasaki disease related syndrome has been shown to be characterized by lymphopenia and overproduction of inflammatory cytokines, albeit with a slightly different (Th17) profile than seen in COVID-19 (Consiglio et al., 2020).

## NK cells in the lungs and airways

The loss of NK cells associated with the observed lymphopenia may be due to apoptosis of these cells, or in part, to trafficking of these cells to the lungs. An analysis of transcripts from cells from bronchoalveolar lavage fluid of COVID-19 patients suggests that

peripheral blood cells (both NK cells and other cell populations) migrate to the lungs. Before discussing the implications of these results, it is important to examine the role of NK cells in the lungs.

In healthy humans, the NK cell population of the lungs is predominantly $CD56^{dim}CD16+$, and the majority of these cells are circulating or derived from recently circulated NK cells. Irrespective of this, the NK cells of the lungs can be differentiated from peripheral blood NK cells. The NK cells in the lungs are more mature, and are characterized by the presence of a terminally differentiated, poorly responsive to cytokines, $CD57^{+}NKG2A^{-}$ subpopulation of $CD56^{dim}$ NK cells that is rarely found in peripheral blood. Despite their $CD56^{dim}$ phenotype, which is normally associated with cytotoxicity, the NK cells found in the lungs (both circulating and a small population of tissue resident cells) are reported to be relatively hyporesponsive (in terms of interferon production and cytotoxicity) compared to other tissue resident and peripheral blood NK cells. However, such as they are, these pulmonary NK cells remain important cytotoxic actors against infected cells and sources of anti-viral IFN-γ in the lung (Cong & Wei, 2019; Robinson et al., 1984). During some acute pulmonary viral infections, the lungs become relatively hypoxic, which may lead to a further inactivation of NK cytolytic activity (Balsamo et al., 2013). However, some infections can modify this balance. In the case of influenza A, for example, a cytotoxic IFN-γ producing $CD56^{bright}CD49a^{+}CD103^{+}CD69^{+}$ NK cell population was found in the lung, with the ability to eliminate virally infected cells (Cooper et al., 2018).

Table 1

*NK roles in COVID-19*

| Potential disease controlling NK effects | Potential disease exacerbating NK effects |
|---|---|
| Killing of virus infected cells via degranulation (liberation of perforin/granulysin/granzyme B) Fas ligand or TRAIL | Exhausted NK cells do not kill, can be pro-inflammatory |
| Rapid production of anti-viral interferons and cytokines such as IFN-γ and TNF-α | Late production of pro-inflammatory cytokines IFN-γ, TNF-α, GM-CSF, induction of cytokine storm |
| Elimination of infected cells that have bound antibody on their surface via ADCC | Killing of bystander lung cells that may have anti-corona virus antibody bound to cell surfaces |
| Early production of growth factors and chemokines to attract monocytes, neutrophils, T, B, cells e.g. CCL3 (MIP-1α), CCL4 (MIP-1β), CCL5 (RANTES), CXCL1 (lymphotoxin), and CXCL8 (IL-8) | Late production of growth factors and chemokines to attract an over active immune response composed of monoctyes, monocyte-derived macrophages, neutrophils, e.g. CCL2, CCL3 (MIP-1α), CCL4 (MIP-1β), CCL5 (RANTES), and CXCL8 (IL-8) |
| Elimination of inflammatory cytokine producing dendritic cells, monocytes and T cells | Exhausted NK cells can not perform this role. Systemic IL-6 blunts NK activity |
| Late production of anti-inflammatory IL-10 or IL-22 assists with regeneration in the lung | Production of IL-10 acts with regulatory T cells to dampen immune response in lungs |
| Production of IFN-γ to induce death receptor expression on virus infected targets | Production of IFN-γ to activate inflammatory monocytes/macrophages and antigen presenting cells leading to lung inflammation |
| Distinct NK subsets in blood and tissue resident NKs control local infections | Respond to infected airway cells or activated neutrophils and traffic to lungs at late stage of disease |
| Mature activated NK cells are cytotoxic and kill infected targets. Usually $CD56^{dim}$ $CD16^{bright}$ NK cells. | Immature NK cells less cytotoxic and produce more proinflammatory cytokines. Often $CD56^{bright}$ NK cells. |

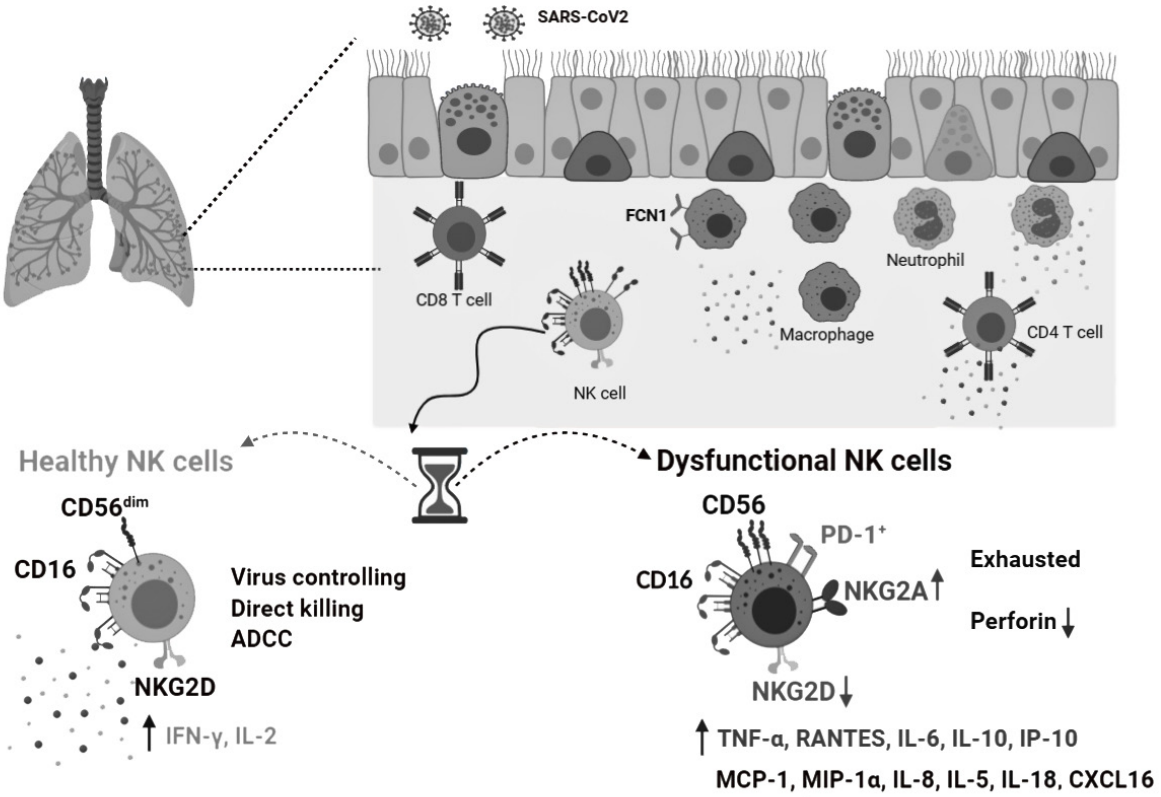


*Figure 1.* Overview of NK cells in COVID-19. Functional antiviral NK cells that exist prior to viral infection are transformed into dysfunctional cells over the course and severity of the disease. Infection in the airways leads to systemic inflammation that may potentiate the change in NK cell activity. Chemotaxis of circulating NK cells to the lungs may further increase dysfunction of NK cells and/or pathologic NK responses in the lungs. Changes in cell populations and inflammatory molecules are some potential targets for therapeutic intervention

This viral clearance, however, may have secondary effects, such as resulting lung pathology. In a mouse model of high dose influenza infection, it was the depletion of NK cells that ameliorated the virus-induced pathology in the lungs (Abdul-Careem et al., 2012). In contrast, other reports show that NK cells, perhaps via IL-22 production (Kumar et al., 2013), are essential for down-regulating pathogenic inflammatory responses and maintaining equilibrium after influenza vaccination (Mooney et al., 2020) and also in diabetes and coronary arterial disease (Gong et al., 2016).

Given these conflicting reports on the roles of NK cells in lung pathologies, the question becomes one of dissecting the roles of timing, trafficking, and different NK subsets. A group working with cell lines, an animal model (ferret) and sub-acute patient samples, found SARS-CoV-2 infection to be characterized by a distinct immune response compared to common respiratory viruses (Blanco-Melo et al., 2020). In patient serum, and similarly in upper respiratory lavages from infected ferrets, this group found a lack of type I and II interferons, and an over-abundance of transcripts for cytokines and chemokines; significant increases were found with respect to IL-6, CCL2 (MCP-1), CCL8, CXCL2, CXCL8 (IL-8), CXCL9, and CXCL16 levels. Notably, CXCL16 and CXCL19 (chemoattractants for NK or T cells, respectively) were significantly upregulated. CCL8 and CCL2 (which recruit monocytes and/or macrophages), and CXCL8 (to recruit neutrophils) were also upregulated, suggesting that the chemotaxis of these cells may be crucial for the pathology observed in the lungs of COVID-19 patients.

Another study investigating severe versus mild COVID-19 cases found that infiltrating monocyte-derived FCN1$^{+}$ inflammatory macrophages dominated the bronchoalveolar lavage fluid of patients with severe COVID-19 disease. These inflammatory macrophages upregulated interferon-signaling genes and monocyte recruiting chemokines including CCL2, CCL3 (MIP-1α), CCL4 (MIP-1β) as well as the cytokines IL-6, IL-8, TNF-α and TGF-β, (Liao et al., 2020). Somewhat contradictorily, in another study, the interferon response was less notable in NK cells found in the bronchoalveolar lavage fluid from severe versus moderate COVID-19 patients, however this group did note increased perforin and granzyme, and noted increased adaptive NK cells and CD56$^{bright}$ NK cells with a more activated and cytotoxic profile in severe versus moderate cases (Maucourant et al., 2020). It is worth noting that the relative increase in the interferon response in moderate patients in the above study may be related to earlier, protective, interferon responses, versus later pathogenic interferon responses seen in other studies with severe patients. In children with severe disease (a relatively rare cohort), the interferon pathway, pro-inflammatory cytokines and activated NK and CD8 T cells were found to be increased in airway cells (Sajuthi et al., 2020).

In contrast to the reports finding migration of NK cells from the blood to the lungs, a study using sequencing from bronchoalveolar lavage found a decrease in resting NK cells, but no changes in activated NK cells in the airways of COVID patients (Z. Zhou et al., 2020). This may be explained by the fact that this group was working with samples taken close to the onset of symptoms, and thus this reinforces the idea that the changes in the NK compartment are time dependent.

In another similar report, single-cell transcriptomes of nasopharyngeal and bronchial samples similarly found that in critical patients, CCL2, CCL3, CCL5 (RANTES), CXCL9, CXCL10, IL-6, IL-1β, IL-8 and TNF-α transcripts were increased in macrophages. Inflammatory macrophages and T cells that were presumed to have trafficked to the lungs were hyperactivated and strongly interacted with epithelial cells (Chua et al., 2020). These inflammatory macrophages are suggested as major sources of IL-6 (Antonioli et al., 2020) and it is notable that increased pulmonary IL-6 has been shown to lead to airway neutrophilia in asthma and colitis (Fu et al., 2013; (Mateer et al., 2018). TNF-α produced by activated macrophages might contribute to differentiation of NK cells and induce IFN production (Almishri et al., 2016). A similar cytokine storm of hyperactive inflammatory responses was observed in SARS-CoV and MERS-CoV with consequential severe lung fibrosis (Azhar et al., 2019; Tseng et al., 2005).

The evidence discussed above raises the question: what is the difference between individuals who are able to rapidly clear SARS-Cov-2 infections, and those who develop the clinical manifestations of severe COVID-19? One possible clue would be the local and systemic cytokine milieus pre-infection. These might very obviously change with age, chronic heart or kidney disease, obesity, diabetes, and other risk factors associated with severe COVID-19 responses. One interesting observation is that, while originally assumed to be a risk factor, allergic asthma, characterized by a Th17 profile, is not currently considered a risk factor, even though this is a disease with a very strong pulmonary component (Chhiba et al., 2020). It is possible that IL-22 produced by Th17 cells is protective in COVID-19, as was seen in influenza infections (Ivanov et al., 2013). Another interesting hypothesis is that a subset of patients may be predisposed to severe COVID-19 due to preexisting high ACE2 expression and low baseline cytotoxic NK and T cell populations in the lung (Duijf, 2020).

## Potential therapeutic applications

Much as the immune response of NK cells in COVID-19 depends on timing and location, so too do the potential therapies that might leverage NK cell biology in the fight against the disease. Currently, this is a topic of great interest which has been amply reviewed by Market et al. (2020). Therapies that are not related or possibly related to NK responses and signaling are not discussed. The potential therapies can be divided into those with potential efficacy in patients in late stage/severe COVID-19, in patients that range from paucisymptomatic to mildly symptomatic, and therapies that might be considered as a general prophylactic in the absence of a vaccine.

For the most severely ill patients, those with respiratory distress and high systemic levels of pro-inflammatory cytokines, the following treatments are considered most promising: (1) Anti-cytokine therapies, such as anti-IL-6R (tocilizumab, sarilumab) (Benucci et al., 2020), anti-RANTES (CCL5) receptor leronlimab (Patterson et al., 2020), anti-IL-8 (clinical trial NCT04347226), anti-GM-CSF (De Luca et al., 2020), Anakinra, the recombinant IL-1-receptor anta-

gonist (IL-1RA) (Huet et al., 2020); or tyrosine kinase inhibitors which would inhibit the downstream effects of cytokine binding and have broad immunosuppressive effects (NIH, 2020), the most promising are baricitinib, a JAK inhibitor, which inhibits the effects of STAT phosphorylation (Cantini et al., 2020) and cytosorb, the mechanical adsorption of cytokines from the patient's blood outside of the body; (2) Anti-inflammatory corticosteroids, such as dexamethasone, hydrocortisone or methylprednisolone (The Writing Committee for the REMAP-CAP Investigators, 2020; Tomazini et al., 2020)(Corral et al., 2020); and (3) Controversially, some would also advocate the use of immune checkpoint inhibitors, such as anti-PD-1 (Di Cosimo et al., 2020) and anti-NKG2A (Yaqinuddin & Kashir, 2020), to reactivate NK and T cells; however, this may be risky in the face of severe inflammation and a cytokine storm.

In the second group, those patients beginning their defense against SARS-CoV2, the use of convalescent serum, or intravenous immunoglobulin, therapies have been widely discussed as a means of rapidly delivering preformed neutralizing antibodies to high risk patients (Casadevall & Pirofski, 2020; Duan et al., 2020). Such therapies may also have a salutary effect on NK cell function and reduce the release of IL-6 by activated inflammatory cells by promoting NK-cell mediated ADCC of activated dendritic cells (Prete et al., 2020). Osman et al. (2020) point out the similarities between the cytokine storm seen in COVID-19 and hemophagocytic lymphohistiocytosis syndrome, where patients are also characterized by the loss of NK effector activity. A similar cytokine storm is also seen in the related Kawasaki disease; and in this disease IVIG therapy was found to augment NKG2D expression, possibly enhancing the perforin pathway and NK cell cytolytic activity (Ge et al., 2013). Similarly, recent preliminary results from a clinical trial with a monoclonal anti-spike protein IgG cloned from patient serum (LY-CoV555, BLAZE-1 Trial) found early administration of this monoclonal antibody to significantly reduce risk of hospitalization when given to mild or moderate patients (ClinicalTrials.gov, 2020).

Additionally, clinical trials have begun with CYNK-001, an NK cell therapy that is developed from placental hematopoietic stem cells (NCT04365101), and with NKG2D-ACE2 Chimeric Antigen Receptor -NK cells (NCT04324996); no results from either of these trials have been reported as of yet. Likewise, exogenous administration of IL-15 has been proposed as a mechanism to hyperactivate the NK response (Kandikattu et al., 2020).

Others have proposed treatments with TGF-β blockers (George et al., 2020). However the results of such a therapy might depend greatly upon time of administration, as early application might assist in activating NK and T cells (via inhibition of Tregs and inhibitory NK cells) but later application may have unforeseen secondary effects on NK cells (Wu et al., 2017). Treg percentages in COVID-19 patients have been shown to correlate inversely with disease severity (Qin et al., 2020). TGF-β has been implicated in lung fibrosis and a significant increase in TGF-β at the later stages of the disease has been reported, raising hopes for therapeutic applications (George et al., 2020). In SARS-CoV-1, lung fibrosis was mediated by the epidermal growth factor receptor, and TGF-β induction drove expression of EGFR ligands (Venkataraman & Frieman, 2017).

In the third group, where therapies would potentially be administered prophylactically, the administration of interferon α, β and λ has been proposed (Acharya et al., 2020; Market et al., 2020). Here, the goal would be to counteract the dysregulated interferon responses that arise as a result of the immunomodulatory strategies used by beta coronaviruses. During the incubation phase, SARS-CoV-2 replicates in host cells and viral products inhibit the interferon response, thus leading to high viral loads; administration of interferon might inhibit subsequent encounters with the virus by priming the innate immune system. Again, timing will be important. Of note is the fact that, in mice, early IFN-β application prior to peak viral replication was protective against MERS-CoV infection, while late IFN administration impeded viral clearance and resulted in increased immunopathology (Channappanavar et al., 2019). Currently clinical trials are underway with several interferon based therapies; while safety and efficacy have not been proven, IFN alfa-2b may be promising (Q. Zhou et al., 2020).

Another prophylactic proposal involves the use of the common diabetes medicine metformin, which has an effect on increasing perforin (controversially, this may increase ACE2 stability), and has been shown to decrease mortality in diabetic COVID 19 patients (Luo et al., 2020).

Strategies to prophylactically activate toll-like receptors (TLRs) or pattern recognition (PRRs) include the administration of heterologous vaccines, such as BCG or similar. The theory behind these treatments is that they would provide early activation to monocyte/macrophages, which would then secrete IL-1β, feeding back to further stimulate the innate response. BCG

has been found to increase perforin production in NK cells (Semple et al., 2011) and re-vaccination in adults boosted NK responses (Suliman et al., 2016).

## Conclusions

While the search for therapies to activate the adaptive immune system (increase the production of neutralizing antibodies), mitigate the cytokine storm and resolve disease in the lungs remain paramount, the role of the innate immune system in the fight against COVID-19 should not be forgotten.

In summary, early in the infection, increased populations of aberrantly activated macrophages may lead to an increased pro-inflammatory environment in the lungs of some patients, which might inhibit the NK response. This could be advantageous, in terms of the pathogenic NK response, but negative in terms of antiviral response and long-term survivability. The ability of different populations of NK cells in the lung to participate in ADCC against virally infected cells is not clear, nor is it certain if such a response would be protective or pathogenic when NK cells migrate to the lung, relatively late in the course of the disease. The roles of unique subsets such as $CD56^{bright}$ or adaptive NK cells have yet to be fully described.

The available evidence leads to the suspicion that the innate immune system might be primed or pre-oriented towards a weaker response in a certain subset of patients. This response may be characterized by the transit of poorly cytotoxic NK cells to the lungs. An alternative explanation is this response may be characterized by the exacerbated levels of IL-6, such that when NK cells arrive and encounter virus infected airway epithelial cells, these NK cells lose cytotoxicity and switch to a more inflammatory profile. The still relevant and not yet completely answered question is the point might this switch occur, and how is it controlled.

In light of the above, when considering new therapies aimed at activating NK cells to fight against COVID-19, care must be taken to evaluate these therapies at different time points in the disease, considering the possibility that re- or hyper- activation of NK cells might have deleterious consequences.

## References

Abdul-Careem, M. F., Mian, M. F., Yue, G., Gillgrass, A., Chenoweth, M. J., Barra, N. G., … Ashkar, A. A. (2012). Critical role of natural killer cells in lung immunopathology during influenza infection in mice. *Journal of Infectious Diseases, 206*(2), 167-177. https://doi.org/10.1093/infdis/jis340

Abel, A. M., Yang, C., Thakar, M. S., & Malarkannan, S. (2018). Natural killer cells: Development, maturation, and clinical utilization. *Frontiers in Immunology, 9*, 1869. https://doi.org/10.3389/fimmu.2018.01869

Acharya, D., Liu, G., & Gack, M. U. (2020). Dysregulation of type I interferon responses in COVID-19. *Nature Reviews Immunology, 20*(7), 397-398. https://doi.org/10.1038/s41577-020-0346-x

Akbar, A. N., & Gilroy, D. W. (2020). Aging immunity may exacerbate COVID-19. *Science*, *369*(6501), 256-257. https://doi.org/10.1126/science.abb0762

Almishri, W., Santodomingo-Garzon, T., Le, T., Stack, D., Mody, C. H., & Swain, M. G. (2016). TNFα augments cytokine-induced NK cell IFNγ production through TNFR2. *Journal of Innate Immunity*, *8*(6), 617-629. https://doi.org/10.1159/000448077

Antonioli, L., Fornai, M., Pellegrini, C., & Blandizzi, C. (2020). NKG2A and COVID-19: Another brick in the wall. *Cellular & Molecular Immunology, 17*(6), 672-674. https://doi.org/10.1038/s41423-020-0450-7

Arentz, M., Yim, E., Klaff, L., Lokhandwala, S., Riedo, F. X., Chong, M., & Lee, M. (2020). Characteristics and outcomes of 21 critically ill patients with COVID-19 in Washington State. *Journal of the American Medical Association, 323*(16), 1612-1614. https://doi.org/10.1001/jama.2020.4326

Arreygue-Garcia, N. A., Daneri-Navarro, A., del Toro-Arreola, A., Cid-Arregui, A., Gonzalez-Ramella, O., Jave-Suarez, L. F., … Del Toro-Arreola, S. (2008). Augmented serum level of major histocompatibility complex class I-related chain A (MICA) protein and reduced NKG2D expression on NK and T cells in patients with cervical cancer and precursor lesions. *BMC Cancer*, *8*(16). https://doi.org/10.1186/1471-2407-8-16

Azhar, E. I., Hui, D. S. C., Memish, Z. A., Drosten, C., & Zumla, A. (2019). The Middle East Respiratory Syndrome (MERS). *Infectious Disease Clinics*

*of North America, 33*(4), 891-905. https://doi.org/10.1016/j.idc.2019.08.001

Balsamo, M., Manzini, C., Pietra, G., Raggi, F., Blengio, F., Mingari, M. C., … Vitale, M. (2013). Hypoxia downregulates the expression of activating receptors involved in NK-cell-mediated target cell killing without affecting ADCC. *European Journal of Immunology,43*(10), 2756-2764. https://doi.org/10.1002/eji.201343448

Benucci, M., Giannasi, G., Cecchini, P., Gobbi, F. L., Damiani, A., Grossi, V., … Manfredi, M. (2020). COVID-19 pneumonia treated with sarilumab: A clinical series of eight patients. *Journal of Medical Virology*, 1-3. 10.1002/jmv.26062. https://doi.org/10.1002/jmv.26062

Bhatraju, P. K., Ghassemieh, B. J., Nichols, M., Kim, R., Jerome, K. R., Nalla, A. K., … Mikacenic, C. (2020). Covid-19 in critically ill patients in the Seattle Region - Case Series. *New England Journal of Medicine*, *382*(21), 2012-2022. https://doi.org/10.1056/NEJMoa2004500

Blanco-Melo, D., Nilsson-Payant, B. E., Liu, W.-C., Uhl, S., Hoagland, D., Møller, R., … tenOever, B. R. (2020). Imbalanced host response to SARS-CoV-2 drives development of COVID-19. *Cell*, *181*(5), 1036-1045.e1039. https://doi.org/10.1016/j.cell.2020.04.026

Bordoni, V., Sacchi, A., Cimini, E., Notari, S., Grassi, G., Tartaglia, E., ... Agrati, C. (2020). An inflammatory profile correlates with decreased frequency of cytotoxic cells in COVID-19. *Clinical Infectious Diseases,* ciaa577. https://doi.org/10.1093/cid/ciaa577

Campisi, J. (2016). Cellular senescence and lung function during aging. Yin and yang. *Annals of the American Thoracic Society*, *13*(Suppl 5), S402-S406. https://doi.org/10.1513/AnnalsATS.201609-703AW

Cantini, F., Niccoli, L., Matarrese, D., Nicastri, E., Stobbione, P., & Goletti, D. (2020). Baricitinib therapy in COVID-19: A pilot study on safety and clinical impact. *Journal of Infection, 81*(12), 318-356. https://doi.org/10.1016/j.jinf.2020.04.017

Casadevall, A., & Pirofski, L. A. (2020). The convalescent sera option for containing COVID-19. *Journal of Clinical Investigation, 130*(4), 1545-1548. https://doi.org/10.1172/JCI138003

Centers for Disease Control and Prevention. (June 30,2020). Interim clinical guidance for management of patients with confirmed Coronavirus disease (COVID-19). Retrieved from https://stacks.cdc.gov/view/cdc/89980

Channappanavar, R., Fehr, A. R., Zheng, J., Wohlford-Lenane, C., Abrahante, J. E., Mack, M., … Perlman, S. (2019). IFN-I response timing relative to virus replication determines MERS coronavirus infection outcomes. *Journal of Clinical Investigation*, *129*(9), 3625-3639. https://doi.org/10.1172/JCI126363

Chhiba, K. D., Patel, G. B., Vu, T. H. T., Chen, M. M., Guo, A., Kudlaty, E., … Peters, A. T. (2020). Prevalence and characterization of asthma in hospitalized and nonhospitalized patients with COVID-19. *Journal of Allergy and Clinical Immunology*, *146*(2), 307-314.e304. https://doi.org/10.1016/j.jaci.2020.06.010

Chua, R. L., Lukassen, S., Trump, S., Hennig, B. P., Wendisch, D., Pott, F., … Eils, R. (2020). *Cross-talk between the airway epithelium and activated immune cells defines severity in COVID-19*. medRxiv. https://doi.org/10.1101/2020.04.29.20084327

Cifaldi, L., Prencipe, G., Caiello, I., Bracaglia, C., Locatelli, F., De Benedetti, F., & Strippoli, R. (2015). Inhibition of natural killer cell cytotoxicity by interleukin-6: Implications for the pathogenesis of macrophage activation syndrome. *Arthritis and Rheumatology, 67*(11), 3037-3046. https://doi.org/10.1002/art.39295

ClinicalTrials.gov. (2020) A Study of LY3819253 (LY-CoV555) and LY3832479 (LY-CoV016) in Participants With Mild to Moderate COVID-19 Illness (BLAZE-1) https://clinicaltrials.gov/ct2/show/NCT04427501?cond=ly-cov555&draw=2

Cong, J., & Wei, H. (2019). Natural killer cells in the lungs. *Frontiers in Immunology*, *10*, 1416. https://doi.org/10.3389/fimmu.2019.01416

Cooper, G. E., Ostridge, K., Khakoo, S. I., Wilkinson, T. M. A., & Staples, K. J. (2018). Human CD49a$^{+}$ lung natural killer cell cytotoxicity in response to influenza a virus. *Frontiers in Immunology*, *9*, A1671. https://doi.org/10.3389/fimmu.2018.01671

Consiglio, C. R., Cotugno, N., Sardh, F., Pou, C., Amodio, D., Zicari, S., ... Brodin, P. (2020). *The immunology of multisystem inflammatory syndrome in children with COVID-19*. medRxiv. https://doi.org/10.1101/2020.07.08.20148353

Corral, L., Bahamonde, A., Delas Revillas, F.A., Gomez-Barquero, J., Abadia-Otero, J., ... Riancho, J.A. (2020). GLUCOCOVID: A controlled trial of methylprednisolone in adults hospitalized with COVID-19 pneumonia. medRxiv 2020.06.17.20133579; https://doi.org/10.1101/2020.06.17.20133579

Cunningham, L., Simmonds, P., Kimber, I., Basketter, D. A., & McFadden, J. P. (2020). Perforin and resistance to SARS coronavirus 2. *Journal of Allergy and Clinical Immunology*, *146*(1), 52-53. https://doi.org/10.1016/j.jaci.2020.05.007

De Luca, G., Cavalli, G., Campochiaro, C., Della-Torre, E., Angelillo, P., Tomelleri, A., … Dagna, L. (2020). GM-CSF blockade with mavrilimumab in severe COVID-19 pneumonia and systemic hyperinflammation: A single-centre, prospective cohort study. (2020). *Lancet Rheumatology, 2*(8), e465-e473. https://doi.org/10.1016/S2665-9913(20)30170-3

Demaria, O., Carvelli, J., Batista, L., Thibult, M. L., Morel, A., André, P., … Vivier, E. (2020). Identification of druggable inhibitory immune checkpoints on Natural Killer cells in COVID-19. *Cellular & Molecular Immunology 17*, 995-997 https://doi.org/10.1038/s41423-020-0493-9

Di Cosimo, S., Malfettone, A., Pérez-García, J. M., Llombart-Cussac, A., Miceli, R., Curigliano, G., & Cortés, J. (2020). Immune checkpoint inhibitors: a physiology-driven approach to the treatment of coronavirus disease 2019. *European Journal of Cancer*, *135*, 62-65. https://doi.org/10.1016/j.ejca.2020.05.026

Diao, B., Wang, C., Tan, Y., Chen, X., Liu, Y., Ning, L., … Chen, Y. (2020). Reduction and functional exhaustion of T cells in patients with coronavirus disease 2019 (COVID-19). *Frontiers in Immunology*, *11*, Article 827. https://doi.org/10.3389/fimmu.2020.00827

Diefenbach, A., & Raulet, D. H. (2001). Strategies for target cell recognition by natural killer cells. *Immunological Reviews*, *181*, 170-184. https://doi.org/10.1034/j.1600-065x.2001.1810114.x

Duan, K., Liu, B., Li, C., Zhang, H., Yu, T., Qu, J., . . . Yang, X. (2020). Effectiveness of convalescent plasma therapy in severe COVID-19 patients. Proceedings of the National Academy of Sciences *U S A, 117*(17), 9490-9496. https://doi.org/10.1073/pnas.2004168117

Duijf, P. H. G. (2020). *Baseline pulmonary levels of CD8+ T cells and NK cells inversely correlate with expression of the SARS-CoV-2 entry receptor ACE2*. bioRxiv. https://doi.org/10.1101/2020.05.04.075291

Fu, J. J., Baines, K. J., Wood, L. G., & Gibson, P. G. (2013). Systemic inflammation is associated with differential gene expression and airway neutrophilia in asthma. *OMICS: A Journal of Integrative Biology*, *17*(4), 187-199. https://doi.org/10.1089/omi.2012.0104

Ge, X., Li, C. R., Yang, J., & Wang, G. B. (2013). Aberrantly decreased levels of NKG2D expression in children with Kawasaki disease. *Scandinavian Journal of Immunology*, *77*(5), 389-397. https://doi.org/10.1111/sji.12022

George, P. M., Wells, A. U., & Jenkins, R. G. (2020). Pulmonary fibrosis and COVID-19: The potential role for antifibrotic therapy. *Lancet Respiratory Medicine*, *8*(8), 807-815. https://doi.org/10.1016/S2213-2600(20)30225-3

Giamarellos-Bourboulis, E. J., Netea, M. G., Rovina, N., Akinosoglou, K., Antoniadou, A., Antonakos, N., … Koutsoukou, A. (2020). Complex immune dysregulation in COVID-19 patients with severe respiratory failure. *Cell Host Microbe*, *27*(6), 992-1000.e1003. https://doi.org/10.1016/j.chom.2020.04.009

Gong, F., Wu, J., Zhou, P., Zhang, M., Liu, J., Liu, Y., … Liu, Z. (2016). Interleukin-22 might act as a double-edged sword in type 2 diabetes and coronary artery disease. *Mediators of Inflammation*, Article 8254797. https://doi.org/10.1155/2016/8254797

He, X., Lau, E. H. Y., Wu, P., Deng, X., Wang, J., Hao, X., . . . Leung, G. M. (2020). Temporal dynamics in viral shedding and transmissibility of COVID-19. *Nature Medicine, 26*(5), 672-675. https://doi.org/10.1038/s41591-020-0869-5

Huet, T., Beaussier, H., & Voisin, O. (2020). Anakinra for severe forms of COVID-19: A cohort study. *Lancet Rheumatology*, *2*(7), e393-e400. https://doi.org/10.1016/ S2665-9913(20)30164-8

Ivanov, S., Renneson, J., Fontaine, J., Barthelemy, A., Paget, C., Fernandez, E. M., … Trottein, F. (2013). Interleukin-22 reduces lung inflammation during influenza A virus infection and protects against secondary bacterial infection. *Journal of Virology*, *87*(12), 6911-6924. https://doi.org/10.1128/JVI.02943-12

Jurado, A., Martin, M. C., Abad-Molina, C., Orduna, A., Martinez, A., Ocana, E., … Molina, J. (2020). COVID-19: age, interleukin-6, C-reactive protein and lymphocytes as key clues from a multicentre retrospective study in Spain. *medRxiv*, 2020.2005.2013.20101345. https://doi.org/10.1101/2020.05.13.20101345

Kandikattu, H. K., Venkateshaiah, S. U., Kumar, S., & Mishra, A. (2020). IL-15 immunotherapy is a viable strategy for COVID-19. *Cytokine Growth Factor Reviews, 54,* 24-31. https://doi.org/10.1016/j.cytogfr.2020.06.008

Kumar, P., Thakar, M. S., Ouyang, W., & Malarkannan, S. (2013). IL-22 from conventional NK cells is epithelial regenerative and inflammation protective during influenza infection. *Mucosal Immunology*, *6*(1), 69-82. https://doi.org/10.1038/mi.2012.49

Laing, A. G., Lorenc, A., Del Molino Del Barrio, I., Das, A., Fish, M., Monin, L., … Hayday, A. (2020). *A consensus Covid-19 immune signature combines immuno-protection with discrete sepsis-like traits associated with poor prognosis*. medRxiv. https://doi.org/10.1101/2020.06.08.20125112

Lanier, L. L., Le, A. M., Civin, C. I., Loken, M. R., & Phillips, J. H. (1986). The relationship of CD16 (Leu-11) and Leu-19 (NKH-1) antigen expression on human peripheral blood NK cells and cytotoxic T lymphocytes. *Journal of Immunology*, *136*(12), 4480-4486.

Li, R., Pei, S., Chen, B., Song, Y., Zhang, T., Yang, W., & Shaman, J. (2020). Substantial undocumented infection facilitates the rapid dissemination of novel coronavirus (SARS-CoV-2). *Science, 368*(6490), 489-493. https://doi.org/10.1126/science.abb3221

Liao, M., Liu, Y., Yuan, J., Wen, Y., Xu, G., Zhao, J., … Zhang, Z. (2020). Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. *Nature Medicine*, *26*(6), 842-844. https://doi.org/10.1038/s41591-020-0901-9

Ljunggren, H. G., & Kärre, K. (1990). In search of the 'missing self': MHC molecules and NK cell recognition. *Immunology Today*, *11*(7), 237-244. https://doi.org/10.1016/0167-5699(90)90097-s

Lu, R., Zhao, X., Li, J., Niu, P., Yang, B., Wu, H., ... Tan, W. (2020). Genomic characterization and epidemiology of 2019 novel coronavirus: Implications for virus origins and receptor binding. *Lancet*, *395*(10224), 565-574. https://doi.org/10.1016/S0140-6736(20)30251-8

Luo, P., Qiu, L., Liu, Y., Liu, X. L., Zheng, J. L., Xue, H. Y., ... Li, J. (2020). Metformin treatment was associated with decreased mortality in COVID-19 patients with diabetes in a retrospective analysis. *American Journal of Tropical Medicine and Hygiene*, *103*(1), 69-72. https://doi.org/10.4269/ajtmh.20-0375

Malhotra, A., & Shanker, A. (2011). NK cells: Immune cross-talk and therapeutic implications. *Immunotherapy*, *3*(10), 1143-1166. https://doi.org/10.2217/imt.11.102

Marcos-Jimenez, A., Sanchez-Alonso, S., Alcaraz-Serna, A., Esparcia, L., Lopez-Sanz, C., … Alfranca, A. (2020). *Deregulated cellular circuits driving immunoglobulins and complement consumption associate with the severity of COVID-19.* medRxiv. https://doi.org/10.1101/2020.06.15.20131706

Market, M., Angka, L., Martel, A. B., Bastin, D., Olanubi, O., Tennakoon, G., … Auer, R. C. (2020). Flattening the COVID-19 curve with natural killer cell-based immunotherapies. *Frontiers in Immunology*, *11*, 1512. https://doi.org/10.3389/fimmu.2020.01512

Mateer, S. W., Mathe, A., Bruce, J., Liu, G., Maltby, S., Fricker, M., … Keely, S. (2018). IL-6 drives neutrophil-mediated pulmonary inflammation associated with bacteremia in murine models of colitis. *American Journal of Pathology*, *188*(7), 1625-1639. https://doi.org/10.1016/j.ajpath.2018.03.016

Maucourant, C., Filipovic, I., Ponzetta, A., Aleman, S., Cornillet, M., Hertwig, L., … Bjorkstrom, N. K. (2020*). Natural killer cell activation related to clinical outcome of COVID-19*. medRxiv, https://doi.org/10.1101/2020.07.07.20148478

Mazzoni, A., Salvati, L., Maggi, L., Capone, M., Vanni, A., Spinicci, M., … Cosmi, L. (2020). Impaired immune cell cytotoxicity in severe COVID-19 is IL-6 dependent. *Journal of Clinical Investigation, 130*(9), 4696-4703. https://doi.org/10.1172/JCI138554

Merad, M., & Martin, J. C. (2020). Pathological inflammation in patients with COVID-19: A key role for monocytes and macrophages. *Nature Reviews Immunology, 20*(6), 355-362. https://doi.org/10.1038/s41577-020-0331-4

Michel, T., Poli, A., Cuapio, A., Briquemont, B., Iserentant, G., Ollert, M., & Zimmer, J. (2016). Human CD56bright NK cells: An update. *Journal of Immunology*, *196*(7), 2923-2931. https://doi.org/10.4049/jimmunol.1502570

Mooney, J. P., Qendro, T., Keith, M., Philbey, A. W., Groves, H. T., Tregoning, J. S., … Riley, E. M. (2020). Natural killer cells dampen the pathogenic features of recall responses to influenza infection. *Frontiers in Immunology*, *11*, 135. https://doi.org/10.3389/fimmu.2020.00135

NIH. (2020). *COVID-19 Treatment Guidelines*. *Kinase Inhibitors: Bruton's Tyrosine Kinase Inhibitors and Janus Kinase Inhibitors. National Institutes of Health*. Retrieved from https://www.covid19treatmentguidelines.nih.gov/immune-based-therapy/immunomodulators/kinase-inhibitors/

Oberfeld, B., Achanta, A., Carpenter, K., Chen, P., Gilette, N. M., Langat, P., … Pillai, S. (2020). SnapShot: COVID-19. *Cell*, *181*(4), 954-954.e951. https://doi.org/10.1016/j.cell.2020.04.013

Oran, D. P., & Topol, E. J. (2020). Prevalence of asymptomatic SARS-CoV-2 infection: A narrative review. *Annals of International Medicine, 173*(5), 362-367. https://doi.org/10.7326/M20-3012

Osman, M. S., van Eeden, C., & Cohen Tervaert, J. W. (2020). Fatal COVID-19 infections: Is NK cell dysfunction a link with autoimmune HLH? *Autoimmunity Reviews*, *19*(7), 102561. https://doi.org/10.1016/j.autrev.2020.102561

Pahl, J. H. W., Cerwenka, A., & Ni, J. (2018). Memory-like NK cells: Remembering a previous activation by cytokines and NK cell receptors. *Frontiers in Immunology*, *9*, 2796. https://doi.org/10.3389/fimmu.2018.02796

Patterson, B. K., Seethamraju, H., Dhody, K., Corley, M. J., Kazempour, K., Lalezari, J. P., … Sacha, J. B. (2020). *Disruption of the CCL5/RANTES-CCR5 pathway restores immune homeostasis and reduces plasma viral load in critical COVID-19*. medRxiv. https://doi.org/10.1101/2020.05.02.20084673

Pesce, S., Greppi, M., Tabellini, G., Rampinelli, F., Parolini, S., Olive, D., … Marcenaro, E. (2017). Identification of a subset of human natural killer cells expressing high levels of programmed death 1: A phenotypic and functional characterization. *Journal of Allergy and Clinical Immunology*, *139*(1), 335-346.e3. https://doi.org/10.1016/j.jaci.2016.04.025

Pinto, D., Park, Y. J., Beltramello, M., Walls, A. C., Tortorici, M. A., Bianchi, S., … Corti, D. (2020). Structural and functional analysis of a potent sarbecovirus neutralizing antibody. *bioRxiv*. https://doi.org/10.1101/2020.04.07.023903

Prete, M., Favoino, E., Catacchio, G., Racanelli, V., & Perosa, F. (2020). SARS-CoV-2 infection complicated by inflammatory syndrome. Could high-dose human immunoglobulin for intravenous use (IVIG) be beneficial? *Autoimmunity Reviews*, *19*(7), 102559-102559. https://doi.org/10.1016/j.autrev.2020.102559

Qin, C., Zhou, L., Hu, Z., Zhang, S., Yang, S., Tao, Y., … Tian, D. S. (2020). Dysregulation of immune response in patients with COVID-19 in Wuhan, China. *Clinical Infectious Diseases, 71,* 762-768. https://doi.org/10.1093/cid/ciaa248

Robinson, B. W., Pinkston, P., & Crystal, R. G. (1984). Natural killer cells are present in the normal human lung but are functionally impotent. *Journal of Clinical Investigation*, *74*(3), 942-950. https://doi.org/10.1172/JCI111513

Sajuthi, S. P., DeFord, P., Jackson, N. D., Montgomery, M. T., Everman, J. L., Rios, C. L., … Seibold, M. A. (2020). *Type 2 and interferon inflammation strongly regulate SARS-CoV-2 related gene expression in the airway epithelium*. bioRxiv. https://doi.org/10.1101/2020.04.09.034454

Semple, P. L., Watkins, M., Davids, V., Krensky, A. M., Hanekom, W. A., Kaplan, G., & Ress, S. (2011). Induction of granulysin and perforin cytolytic mediator expression in 10-week-old infants vaccinated with BCG at birth. *Clinical and Developmental Immunology*. https://doi.org/10.1155/2011/438463

Shi, H., Wang, W., Yin, J., Ouyang, Y., Pang, L., Feng, Y., … Chen, D. (2020). The inhibition of IL-2/IL-2R gives rise to CD8. *Cell Death & Disease*, *11*(6), 429. https://doi.org/10.1038/s41419-020-2636-4

Spurrell, J. C., Wiehler, S., Zaheer, R. S., Sanders, S. P., & Proud, D. (2005). Human airway epithelial cells produce IP-10 (CXCL10) in vitro and in vivo upon rhinovirus infection. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *289*(1), L85-95. https://doi.org/10.1152/ajplung.00397.2004

Stokic-Trtica, V., Diefenbach, A., & Klose, C. S. N. (2020). NK Cell Development in Times of Innate Lymphoid Cell Diversity. *Frontiers Immunology*, *11*, Article 813. https://doi.org/10.3389/fimmu.2020.00813

Suliman, S., Geldenhuys, H., Johnson, J. L., Hughes, J. E., Smit, E., Murphy, M., … Scriba, T. J. (2016). Bacillus Calmette-Guérin (BCG) revaccination of adults with latent *Mycobacterium tuberculosis* infection induces long-lived BCG-reactive NK cell responses. *Journal of Immunology*, *197*(4), 1100-1110. https://doi.org/10.4049/jimmunol.1501996

Tan, M., Liu, Y., Zhou, R., Deng, X., Li, F., Liang, K., & Shi, Y. (2020). Immunopathological characteristics of coronavirus disease 2019 cases in Guangzhou, China. *Immunology*, *160*(3), 261-268. https://doi.org/10.1111/imm.13223

The Writing Committee for the REMAP-CAP Investigators. (2020). Effect of hydrocortisone on mortality and organ support in patients with severe COVID-19: The REMAP-CAP COVID-19 corticosteroid domain randomized clinical trial. *Journal of the American Medical Association, 324*(13), 1317-1329. https://doi.org/10.1001/jama.2020.17022

To, K. K., Tsang, O. T., Leung, W. S., Tam, A. R., Wu, T. C., Lung, D. C., … Yuen, K. Y. (2020). Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: An observational cohort study. *Lancet Infectious Diseases*, *20*(5), 565-574. https://doi.org/10.1016/S1473-3099(20)30196-1

Tomazini, B. M., Maia, I. S., Cavalcanti, A. B., Berwanger, O., Rosa, R. G., Veiga, V. C., . . . Coalition COVID-19 Brazil III Investigators. (2020). Effect of dexamethasone on days alive and ventilator-free in patients with moderate or severe acute respiratory distress syndrome and COVID-19: The CoDEX randomized clinical trial. *Journal of the American Medical Association, 324*(13), 1307-1316. https://doi.org/10.1001/jama.2020.17021

Tokunaga, R., Zhang, W., Naseem, M., Puccini, A., Berger, M. D., Soni, S., … Lenz, H. J. (2018). CXCL9, CXCL10, CXCL11/CXCR3 axis for immune activation - A target for novel cancer therapy. *Cancer Treatment Reviews*, *63*, 40-47. https://doi.org/10.1016/j.ctrv.2017.11.007

Tseng, C. T., Perrone, L. A., Zhu, H., Makino, S., & Peters, C. J. (2005). Severe acute respiratory syndrome and the innate immune responses: modulation of effector cell function without productive infection. *Journal of Immunology*, *174*(12), 7977-7985. https://doi.org/10.4049/jimmunol.174.12.7977

Varchetta, S., Mele, D., Oliviero, B., Mantovani, S., Ludovisi, S., Cerino, A., … Mondelli, M. U. (2020). Unique immunological profile in patients with COVID-19. Research Square. https://doi.org/10.21203/rs.3.rs-23953/v1

Venkataraman, T., & Frieman, M. B. (2017). The role of epidermal growth factor receptor (EGFR) signaling in SARS coronavirus-induced pulmonary fibrosis. *Antiviral Research*, *143*, 142-150. https://doi.org/10.1016/j.antiviral.2017.03.022

Vitale, M., Cantoni, C., Della Chiesa, M., Ferlazzo, G., Carlomagno, S., Pende, D… Sivori, S. (2019). An historical overview: The discovery of how NK cells can kill enemies, recruit defense troops, and more. *Frontiers in Immunology*, *10*, 1415. https://doi.org/10.3389/fimmu.2019.01415

Vivier, E., Tomasello, E., Baratin, M., Walzer, T., & Ugolini, S. (2008). Functions of natural killer

cells. *Nature Immunology*, *9*(5), 503-510. https://doi.org/10.1038/ni1582

Wang, Y., Liao, B., Guo, Y., Li, F., Lei, C., Zhang, F., ... Guan, Y. (2020). Clinical characteristics of patients infected with the novel 2019 Coronavirus (SARS-Cov-2) in Guangzhou, China. *Open Forum Infectious Diseases*, *7*(6), ofaa187. https://doi.org/10.1093/ofid/ofaa187

Wilk, A. J., Rustagi, A., Zhao, N. Q., Roque, J., Martínez-Colón, G. J., McKechnie, J. L., … Blish, C. A. (2020). A single-cell atlas of the peripheral immune response in patients with severe COVID-19. *Nature Medicine*, *26*(7), 1070-1076. https://doi.org/10.1038/s41591-020-0944-y

Wu, J., Gao, F. X., Wang, C., Qin, M., Han, F., Xu, T., … Dai, T. Y. (2019). IL-6 and IL-8 secreted by tumour cells impair the function of NK cells via the STAT3 pathway in oesophageal squamous cell carcinoma. *Journal of Experimental & Clinical Cancer Research*, *38*(1), 321. https://doi.org/10.1186/s13046-019-1310-0

Wu, Y., Tian, Z., & Wei, H. (2017). Developmental and functional control of Natural Killer cells by cytokines. *Frontiers in Immunology*, *8*, 930. https://doi.org/10.3389/fimmu.2017.00930

Yaqinuddin, A., & Kashir, J. (2020). Innate immunity in COVID-19 patients mediated by NKG2A receptors, and potential treatment using Monalizumab, Cholroquine, and antiviral agents. *Medical Hypotheses*, *140*, 109777. https://doi.org/10.1016/j.mehy.2020.109777

Zeng, X., Moore, T. A., Newstead, M. W., Deng, J. C., Lukacs, N. W., & Standiford, T. J. (2005). IP-10 mediates selective mononuclear cell accumulation and activation in response to intrapulmonary transgenic expression and during adenovirus-induced pulmonary inflammation. *Journal of Interferon & Cytokine Research*, *25*(2), 103-112. https://doi.org/10.1089/jir.2005.25.103

Zheng, H. Y., Zhang, M., Yang, C. X., Zhang, N., Wang, X. C., Yang, X. P., … Zheng, Y. T. (2020). Elevated exhaustion levels and reduced functional diversity of T cells in peripheral blood may predict severe progression in COVID-19 patients. C*ellular & Molecular Immunology*, *17*(5), 541-543. https://doi.org/10.1038/s41423-020-0401-3

Zheng, M., Gao, Y., Wang, G., Song, G., Liu, S., Sun, D., … Tian, Z. (2020). Functional exhaustion of antiviral lymphocytes in COVID-19 patients. C*ellular & Molecular Immunology*, *17*(5), 533-535. https://doi.org/10.1038/s41423-020-0402-2

Zhou, Q., Chen, V., Shannon, C. P., Wei, X. S., Xiang, X., Wang, X., … Fish, E. N. (2020). Interferon-α2b treatment for COVID-19. *Frontiers in Immunology*, *11*, 1061. https://doi.org/10.3389/fimmu.2020.01061

Zhou, Z., Ren, L., Zhang, L., Zhong, J., Xiao, Y., Jia, Z., … Wang, J. (2020). Heightened innate immune responses in the respiratory tract of COVID-19 patients. *Cell Host Microbe*, *27*(6), 883-890.e882. https://doi.org/10.1016/j.chom.2020.04.017