

# Discuss and Envision the Immunotherapy Potential based on NK Cell to Treat COVID-19

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**Abstract:** Corona virus disease 2019(COVID-19), caused by SARS-COV-2, has posed a tremendous threat to humans and public health worldwide. When faced with viral infection, our immune systems need to achieve a precise balance to eliminate the pathogen. In most cases, however, this balance is disturbed and then further distorted by viral infection. As an indispensable part in innate immune system, NK cells play a pivotal role in immune defense, immune surveillance, and immune homeostasis. Furthermore, NK cells showcase an effective capability of lysing virally infected cells and regulating immune responses. It has been demonstrated that COVID-19 patients with severe symptoms manifest a reduction in NK cell number as well as its function, which contributes to decreased clearance of infected cells. Restoration of NK cells have the potential to calibrate this delicate balance entailed to eliminate SARS-COV-2 infection. Based on current clinical studies, the immunopathologies of COVID-19 patients are summarized in this article. In addition, NK cell immune responses to COVID-19, factors concerning the immune responses of NK cell, and relevant feasible immunotherapies are also reviewed.

**Keywords:** Novel Coronavirus; COVID-19; NK Cell; Immunotherapy.

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## 1. Introduction

At the end of 2019, COVID-19 pneumonia suddenly spread rapidly. ZHU et al. were the first to isolate and identify the pathogenic virus - severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) - in bronchoalveolar lavage fluid (BALF) samples from patients. With the continuous progress of research, it has been found that COVID-19 patients have immune system disorders, especially with decreased lymphocytes and increased inflammatory response, which are closely related to the severity of the disease.[1]

The coronavirus is elliptical or spherical in shape and belongs to a type of single stranded positive RNA virus. The virus has an outer envelope with spikes on it. The coronavirus is transmitted through the respiratory tract and can also be transmitted through airborne droplets. The S protein on the surface of SARS-COV-2 virus exhibits extensive glycosylation, which binds to the host cell ACE2 receptor and enters the host cell for proliferation and diffusion.[2]

NK cells can effectively regulate the activity of the immune system and exert significant antiviral effects. It is increasingly clear that tissue localization is a key factor in determining the immune function and in vivo effects of lymphocytes. Improving the level and activity of NK cells in the patient's body, enhancing the body's innate immune function, has promising prospects in the prevention and treatment of COVID-19. [1]

## 2. The Role of NK Cell Immune Regulation

NK cells, as innate circulating lymphocytes, constitute a part of the body's innate immunity. NK cells are not only the first line of defense against pathogens, but also have the ability to regulate adaptive immune responses. NK cells mainly clear aging, viral infections, and mutated cells in the body, and have their independent functions to exert cellular activity without the need for pre sensitization. Consistent with

T cells and B cells, NK cells develop from lymphoid progenitor cells and recognize specific ligands expressed on host cells through lineage encoding inhibition and activation receptors. [3] NK cell activation causes cytotoxic degranulation, produces inflammatory cytokines, and subsequently kills target cells. At the same time, NK cells do not damage the body when they function, and they respond quickly to external stimuli with high recognition and intensity. The biological activity of NK cells also helps to activate immune cells such as T cells and B cells, enhancing the overall immune level of the body. Research has found that when NK cells are exposed to SARS-COV-2 antigen again, their dependence on CD4+T cells is enhanced. This reaction is related to T cells, SARS-COV-2 specificity, and the number of CD4+T cells; Moreover, interleukin (IL-2) is an important mediator that affects the function of NK cells. [4] The MHC I (major histocompatibility complex class I) molecule in normal body tissue cells binds to the killer cell immunoglobulin like receptor (KIR) on the surface of NK cells, labeling healthy cells as "self" and generating inhibitory signals to prevent NK cells from killing their own healthy cells. [2]

In addition, NK cells play an equally important role in immune balance regulation. NK cells can inhibit overactivated immune cells, especially by regulating the activity of T cells. In addition to T cells, NK cells can also regulate immune activity such as neutrophils and monocyte macrophage systems, regulate immune response balance, avoid immune pathological damage, and reduce the severity of viral infection. [1] NK cells can also indirectly regulate immune balance by interacting with antigen-presenting cells (such as dendritic cells) or directly interacting with T cells themselves. During acute infection, NK cells secrete and release interferon  $\gamma$  (Inferon)-  $\gamma$ , IFN-  $\gamma$ , Promoting the differentiation of CD4+T cells into Th1 T cells can enhance the ability to control pathogens. NK cells can also inhibit T cell maturation through IL-10, thereby regulating T cell activity.

Cellular metabolism is equally crucial for the normal function of immune cells, which rely on energy to exert immune efficacy. Once activated, NK cells undergo highly characteristic metabolic changes to meet their energy needs. Glycolysis and oxidative phosphorylation (OXPHOS) are both important for NK cell metabolism, and NK cells are more inclined towards glycolysis because glycolysis provides energy at a faster rate and can also compensate for other metabolic pathways. When the deficiency of cyclooxygenase-2 (COX2) leads to OXPHOS deficiency, the expression of related glycolytic enzyme genes, such as enolase 1 (ENO1) and aldolase A (ALDOA), is upregulated. Relevant studies have found that in COVID-19 patients, the level of some metabolic pathways is elevated, such as glycolysis, phosphoinositol and glycerol ester metabolism. These metabolic changes may enhance NK cell function, such as chemotaxis, degranulation, and cytotoxicity.

### 3. NK Cell Subpopulations and Antiviral Mechanisms

#### 3.1. NK Cell Subpopulations

NK cells can be divided into two subgroups: CD56<sup>dim</sup> and CD56<sup>bright</sup>. CD56<sup>dim</sup>CD16<sup>+</sup>NK cells are abundant in peripheral blood, exhibiting cytotoxicity and primarily killing function. They express perforin and produce IFN- $\gamma$ . Ability; CD56<sup>bright</sup>CD16<sup>-</sup> exists in lymphoid tissue and plays an immune regulatory role. It lacks the ability to express perforin, but can produce IFN- $\gamma$ . Wait for cytokines to respond to the stimulation of IL-12, IL-15, and IL-18, thereby enhancing the NK cell effect. [6] There is also a CD56<sup>neg</sup>CD16<sup>+</sup>NK cell subpopulation in peripheral blood, which is related to NK cell dysfunction in pathological conditions. [1]

#### 3.2. NK Cell Antiviral Mechanism

NK cells can exert killing effects through various mechanisms: after recognizing virus infected cells, NK cells can quickly release perforin and granular enzymes to lyse target cells. Some cytokines, such as IL-2, IL-12, IL-15, etc., can induce NK cells to release pro-inflammatory cytokines to indirectly clear target cells, including IFN- $\gamma$ . And tumor necrosis factor  $\alpha$  (tumor necrosis factor)- $\alpha$ , TNF- $\alpha$ ). Activate a broader immune response, control viral infection, or allow NK cells to express CD16 to recognize target cells coated with antibodies, exerting antibody dependent cell mediated cytotoxicity (ADCC), directly or indirectly clearing virus-infected cells. NK cells can also indirectly recognize "non self" cells and Toll like receptor (TLR) ligands by interacting with monocytes, inducing the production of IFN- $\gamma$ . Enhance cytotoxicity. [6] In addition, NK cells also have the function of immune memory. After viral infection, NK cells can survive for a long time and retain specific antigen memory. After secondary infection, their effectiveness in combating pathogens is more significant. [1]

### 4. Immunopathological Status of Novel Coronavirus

After infection with SARS-COV-2, there is an abnormal synergistic effect between innate immunity and adaptive immunity, and one of the main immunopathological features is a general decrease in lymphocytes. In critically ill patients, the decrease in CD8<sup>+</sup> and NK cells is particularly significant. The expression of natural killer group 2 member A (NKG2A)

on the surface of NK cells is upregulated, and IFN is secreted- $\gamma$ , IL-2, TNF- $\alpha$ . The ability decreases, and the expression level of Granzyme B decreases, leading to a weakened ability to resist infections and a weakened immune system. The decrease in NK cell count is related to the intensity of inflammation, which may be caused by virus induced cell apoptosis, as SARS-COV-2 has the ability to enhance CD-95 expression levels. [7] It can be seen that the decrease in the number of cytotoxic lymphocytes is directly related to the severity of the disease. In addition, in critically ill patients, there is a sharp increase in pro-inflammatory cytokines such as IL-2, IL-7, IL-10, and granulocyte colony stimulating factor (G-CSF). IL-6 is one of the most critical factors causing cytokine release syndrome (CRS), and high levels of IL-6 release can inhibit NK cell toxicity and downregulate the release of perforin and granzyme. It is worth mentioning that during viral infection, CXCR3<sup>+</sup>NK is more common in the CD56<sup>bright</sup> subgroup when NK cell subsets are imbalanced, indicating that NK cell imbalance is more inclined towards inflammation rather than cytotoxicity; In addition, studies have found a negative correlation between serum IL-6 levels and the frequency of Granzyme A expression in NK cells. [6] Clinical studies have shown that viral infections can cause systemic inflammation, hemodynamic instability, methemoglobinemia, and cytokine storm syndrome (CSS) in patients. [2]

### 5. Factors Influencing Abnormal NK Cell Function

#### 5.1. Age Related Immune Aging

Aging is associated with the development of chronic inflammation and a decrease in overall immune levels. In the innate immune response of aging, increased secretion of pro-inflammatory cytokines is the main feature, including TNF, IL-6, and IL-1 $\beta$ ; Moreover, it is accompanied by a decrease in the number and function of dendritic cells and macrophages, leading to impaired T cell maturation and weakened phagocytic clearance of infected and apoptotic cells.

The production and proliferation of NK cells decrease with age, although the absolute count of NK cells increases, which may be due to the accumulation of long-lived NK cells. During the aging process, the number of NCR and NKp30 decreases, which not only reduces particle mediated cytotoxicity, but also has a negative impact on adaptive immunity by blocking NK-DC cross-linking crosstalk. The composition of NK cell subpopulations in elderly individuals shows a higher number of mature NK cells, and an increase in the ratio of CD56<sup>dim</sup> to CD56<sup>bright</sup>. The increase in CD56<sup>dim</sup> cells may be a compensatory effect on the reduction of NK cell toxicity in elderly individuals. [6]

#### 5.2. Effects of Mg<sup>2+</sup> Levels on NK Cell Function

In the patient's body, a decrease in intracellular free Mg<sup>2+</sup> will lead to a decrease in NK cell surface activated receptors NKG2D and CD8<sup>+</sup>T lymphocytes, resulting in a weakened immune system's resistance to viruses. It is speculated that the lack of Mg<sup>2+</sup> is an important reason for the aggravation of COVID-19. [9] During viral infection, the number of NK cells in the patient's body decreases, which is consistent with the high expression of immune cell surface inhibitory receptors (such as PD-1, T cell immunoglobulin

mucin 3 (Tim-3), NKG2A, etc.), as well as the low expression of activated receptors (such as NKG2A, CD38, etc.). Among them, NKG2D has a wide range of activation abilities by binding to a large number of ligands. The decrease in NKG2D expression and function will lead to virus escape, increased expression of pro-inflammatory cytokines, and increased pulmonary pathological reactions. Through the lymphocyte receptor pathway, Mg<sup>2+</sup> can act as a second messenger to regulate the antiviral activity of lymphocytes. It has been confirmed that the level of Mg<sup>2+</sup> in the serum of COVID-19 patients is positively correlated with the expression of the activated receptor NKG2D, and negatively correlated with the expression level of the PD-1 receptor on the surface of NK cells. [9]

### **5.3. Atherosclerosis and Cardiovascular Diseases**

Atherosclerosis can cause a series of immune related reactions. The course of the disease has been characterized by macrophages derived from monocytes. The pathogenic T cells in atherosclerosis have the characteristics of Th1 and produce proinflammatory cytokines, such as IFN- $\gamma$ , and activate macrophages. In addition, oxidized low-density lipoprotein produces an inflammatory response by binding to TLR.

Atherosclerotic plaques are relatively stable and are less likely to cause symptoms, but when they become unstable, vascular complications may increase. Research has shown that the subpopulation of CD56BRIGHT NK cells increases in plaques; In addition, as a ligand for activating receptor NKG2D, MICA/B is also expressed in plaques. Chronic giant viral infection (CMV) can also highly activate NK cells, leading to increased expression of the NK cell activating receptor NKG2C and worsening of inflammatory symptoms. [6]

## **6. The "Double-edged Sword" Effect of NK Cells in Antiviral Therapy**

During viral infection, NK cells may have a double-edged sword effect. On the one hand, NK cells can eliminate pathogens and maintain the body's homeostasis; Overactivated NK cells also exhibit a negative side, as their high reactivity can lead to tissue damage. NK cells in bronchoalveolar lavage fluid, trachea, and lung tissue can secrete IL-22, which is beneficial for epithelial cell regeneration. In addition, in IAV infected mice, NK cells exhibit cytotoxicity and secrete IFN- $\gamma$ , Stimulate adaptive cells to clear viruses. However, overactivated NK cells may cause immunopathological damage. Studies have shown that a decrease in the number of NK cells alleviates the lung immune pathology in mice infected with high-dose IAV virus, indicating that NK cells exacerbate the immune pathological damage caused by high-dose virus infection. [10] In addition, in the lungs of influenza virus infected mice, the adoption and transfer of NK cells from them can cause faster weight loss and increased lethality. Compared with mild patients, patients with severe infections may have excessive activation of NK cells by IL-6 and IL-18, which is not conducive to the development of the disease. [11] From this, it can be seen that in viral infection, NK cells have complex and variable functions, which are influenced by viral infection factors and doses, leading to NK cells exhibiting a "double-edged sword" effect of fighting against viruses and exacerbating tissue

immune pathological damage.

## **7. NK Cell Immunotherapy Regimen for COVID-19**

NK has shown excellent abilities in inhibiting and clearing viruses, making the prospect of using NK cell therapy to prevent and treat COVID-19 broad, and people have high expectations for it. NK cells and chimeric antigen receptor NK cells (CAR-NK) are the main candidates for this therapy.

### **7.1. NK Cell Self Proliferation Activation and Related Strategies**

Research has confirmed that vitamin C can effectively promote the in vitro expansion of NK cells, enhance Nkp46, CD69, and IFN- $\gamma$  Equal expression levels enhance NK cell immune activity. [12] In the patient's body, IL-6 is one of the key cytokines that trigger CRS and can also have a negative effect on NK cell activity. Therefore, IL-6 receptor inhibitor tocilizumab can be used for treatment; [13] Studies have shown that flavonoids quercetin and luteolin can reduce the expression level of IL-6 in mast cells, restore NK cell cytotoxicity, and enhance immune response, which may become a safer alternative to corticosteroid therapy. In addition, the role of anti-inflammatory cytokines cannot be ignored. Although no human experiments have been conducted, IL-37 can inhibit the systemic inflammatory response in influenza mouse models. Therefore, IL-37 has potential advantages in treating COVID-19. In NK cell therapy, IL-15 super agonists and granulocyte macrophage colony-stimulating factor (GM-CSF) can also be used to neutralize NKG2D-ACE2 CAR-NK secreting scfv in umbilical cord blood. This treatment method can block the infection of SARS-CoV-2 in ACE2 presenting cells and enhance the cytotoxicity of NK cells. Finally, another factor that enhances the efficacy of NK cells is physical exercise. Insufficient physical exercise will reduce NK cell activity and IFN- $\gamma$  Strengthening physical exercise, especially among high-risk populations, may have a positive effect on the treatment of COVID-19. [6]

### **7.2. NK Cell Adoptive Immunotherapy**

Chimeric antigen receptor NK cells significantly enhance the cytotoxicity and targeting of NK cells, and do not cause an increase in cytokine levels such as IL-6, leading to CRS. In addition, the surface activated receptor NKG2D on NK cells can effectively enhance their ability to recognize target cells, increase IL-15 expression levels, enhance immune activity, promote cell proliferation, and prolong the immune function of CAR-NK in vivo. It can also secrete CM-CSF neutralizing antibodies to delay or prevent the occurrence of CRS. [10] The combination of CAR principles determines the specificity of targeting antigens. NK cells with CAR specific gene modifications can be used in COVID-19 treatment, and CAR-NK has been proven to be an "off the shelf" allogeneic product that can be used to treat various diseases. [14]

NK cell-mediated ADCC may be one of the key mechanisms for alleviating patient symptoms and improving outcomes. [15] Adaptive NK cells have the ability to enhance ADCC, partly due to epigenetic control of signaling molecule Fc  $\epsilon$  RI- $\gamma$  Downregulation of. Related research reports show that adaptive NK cells can effectively clear EBV and HSV infected cells through ADCC mediation. [16] Under normal circumstances, MHC class I molecules present in normal

tissue cells bind to the surface receptor KIR of NK cells to generate inhibitory signals, thereby avoiding NK cells from accidentally damaging normal cells. At present, a major challenge in treatment is the lack of specific drugs. The use of NK cell transfusion therapy has great potential and is a feasible treatment option. There have also been multiple NK cell transfusion test projects carried out, such as the Guangdong Province Umbilical Cord Blood Hematopoietic Stem Cell Bank using a treatment plan combining umbilical cord blood NK cells with umbilical cord blood mesenchymal stem cells. [2] In addition, although NK cells have historically been classified as innate immune cells, it is now believed that NK cells have certain memory or memory like immune response abilities, in which effector cells expand under antigen stimulation and produce long-lived memory cells with enhanced functions. [17] The continuous expansion of NK memory cells, characterized by activated phenotype in response to viral infection, is beneficial for future vaccine development and NK cell adoption therapy for COVID-19. [18] The balance of innate immunity, cellular immunity, and humoral immunity, as well as the moderate release of viral antibodies, are key factors in ensuring the clearance of SARS-COV-2. During the rehabilitation period, these immune response characteristics in the patient's body may provide evidence for immune response monitoring and clinical treatment plans. [19]

## 8. Summary and Outlook

Novel coronavirus is still spreading all over the world, which is particularly harmful to the human immune system. In COVID-19 patients, the number of immune cells is reduced, the function is reduced, and the immune system is in disorder. NK cells, as the first line of defense against viruses, play a crucial role in maintaining immune homeostasis. Related treatment options, such as using vitamin C, tocilizumab, etc., have broad prospects, and the use of NK cell adoptive therapy or NK cell cord blood transfusion also has great potential. However, for severe or critically ill patients, excessive NK cell activity or overexpression of pro-inflammatory cytokines may cause damage to lung tissue, even leading to acute respiratory distress syndrome (ARDS) or severe immunopathological damage. Further research on NK cells, such as biological characteristics or drug development, to enhance their immune activity may have milestone significance in this pandemic battle.

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