



NK CELLS AND COVID19-A KILLER APPROACH

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Abstract: Natural Killer cells (NK Cells) act as the first line of defense against viral propagation. Being key players of innate immunity, they help in controlling viral propagation. In the ongoing COVID 19 pandemic scenario, Severe Acute Respiratory Syndrome Coronavirus2 [SARS COV-2] is known to infect humans. Following infection, viral replication machinery in order to maintain functional integrity, tweaks the host immune system in its favor. Even though the molecular tinkering might not completely render the virus immune-proof to the host immune system, but buys time to allow its multiplication and spread. SARS-COV-2 infected patients develop severe and uncontrolled immune response. This is associated with increase in several cytokines and migration of immune cells to the affected area.

NK Cells of innate immunity are known to be attracted towards and kill an infected cell without much antigenic specificity. SARS COV2 evades first line-of-defense by inactivating NK Cells. The Spike Protein1 (SP-1) on the virus when presented by HLA-E, interacts with the inhibitory receptor CD94/NKG2A on NK cells to inactivate it, preventing destruction of the infected cells. Crosstalk between innate and adaptive immunity is also prevented as active NK cell stimulates TH1 (T-helper 1) cells expansion via induction of IL-12 by macrophage.

Therapeutic intervention with NK cells in hyper-activated inflammatory COVID patient might be a significant strategy. The CD94/NKG2A receptor [C type lectin receptor that recognize carbohydrate moiety] can be used as a plausible target for carbohydrate moieties (of the virus), thereby preventing interaction with HLA-E and thus switching off inhibitory signals. Antibody dependent cell mediated cytotoxicity may also be a probable way. In this case, mAb (monoclonal antibodies) designed against SP1 protein can be produced; Fab of the mAb region binds to displayed SP-1 peptide by HLA-E, and Fc region of the mAb binds to CD16A on NK cells and destroy the infected cell.

Keywords: SARS-CoV-2, NK cells, HLA-E, CD94/NKG2A receptor

I. INTRODUCTION

The first case of COVID 19 was reported in Wuhan city in China during December 2019. [1,2] The original spread was known to have started from non-human source to a human, marking it to be a zoonotic disease. It has increased to an epidemic and then to a pandemic level threat since then. Severe Acute Respiratory Syndrome Corona virus 2 (SARS-COV-2) causes severe respiratory distress. In the past two decades coronavirus has caused other epidemic diseases Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) [3-7]. According to latest data as of now and upto 25th June 2020, the number of confirmed cases is 9,296,202 and number of deaths is 479,133 around the world, reported by WHO. In India, from Jan 30 to 2:25pm CEST to 25 June 2020, there have been 473,105 confirmed cases of COVID-19 with 14,894 deaths (WHO Report).

II. CORONA VIRUS FAMILY

The corona virus is present for two decades now. Coronaviruses belong to the Genus *Betacoronavirus* in the family *Coronaviridae*[8]. A Corona virus typically affects the respiratory tracts of birds and mammals, including humans. Doctors associate them with the common cold, bronchitis, pneumonia, severe acute respiratory syndrome (SARS), and coronavirus disease 2019 (COVID-19). These viruses can also affect the gut. First coronavirus was identified in 1937, which was responsible for a type of bronchitis in birds[9]. Scientists found evidence of human corona viruses in the 1960s, in the noses of people with the common cold[10]. Human corona viruses that are particularly prevalent include 229E, NL63, OC43, and HKU1[11]. The name “coronavirus” comes from the crown-like projections on their surfaces. “Corona” in Latin means “halo” or “crown.” Corona virus spreads by droplets coming out during cough and sneeze, or touching an area infected with virus and then touching eyes nose and ears with that hand[12]. Recently further 3 types of coronavirus was reported; Severe Acute Respiratory Syndrome CoV (SARS-CoV) reported in November 2002 in Guangdong province in China; Middle East Respiratory Syndrome CoV (MERS-CoV) was reported in 2012 in Saudi Arabia. The most recent type being SARS-CoV2 responsible for the current pandemic situation was first reported in 2019 December. The common symptoms of all corona viruses are breathlessness, fever, cough, runny nose, sore throat in mild cases. Along with the common symptom SARS-CoV2 infected patients develop diarrhea, loss of smell and taste, muscle and body aches. **Figure.1** gives a brief synopsis about the different clinical phases that has been observed in COVID cases. COVID-19 presents with three stages of diseases. It starts with upper respiratory disease symptoms followed by pneumonia and disseminated inflammation, and in the third-stage cytokine storm, sepsis-like syndrome, and multiorgan failure take place. The disease that moves into second phase always carries the risk for secondary bacterial infections. Antiviral immune response and tissue and systemic inflammation are different in all stages.

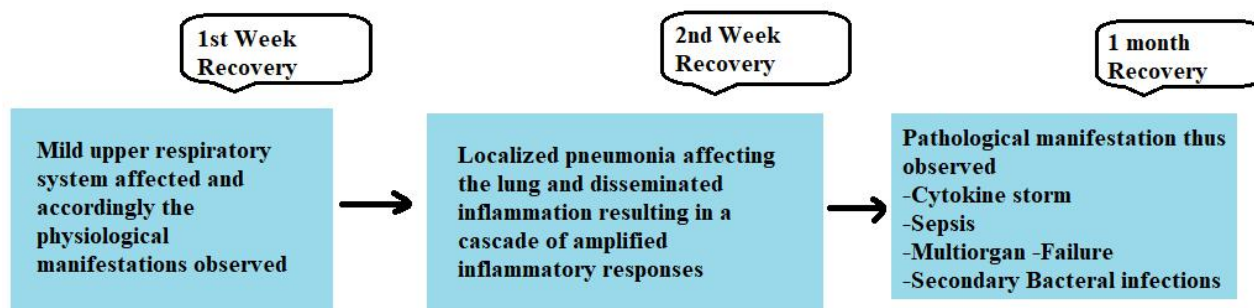


Figure 1: Different clinical phases in COVID cases

III. ENTRANCE OF SARS COV2 INTO HOST CELL

The virus enters the host cell by binding to receptors; Angiotensin Converting Enzyme 2 (ACE2) receptor. The virus has spike proteins on its surface that binds to the ACE2 receptor and facilitate the release of genome inside the host cell[13]. The structural proteins present on the surface of virus are - (S) glycoprotein, small envelope, (E) glycoprotein, membrane (M) glycoprotein, and nucleocapsid (N) protein, and also several accessory proteins. The S glycoprotein or Spike protein has 2 subunits; S1 and S2 of which S2 mediates virus fusion in transmitting host cell and S1 is responsible for host virus range and cellular tropism [13]. ACE2 receptor is present in heart, lung, kidney and gastrointestinal tract allowing virus entry. The attachment occurs between S protein and ACE-2 receptor, followed by fusion of viral membrane and host cell membrane. Following fusion Type II Transmembrane Serine Protease (TMPRSS2) cleaves and activates S protein, leading to a conformational change allowing virus to enter cell. SARSCoV2 releases its genome into the cytoplasm and become translated in nucleus. **Figure.2** summarizes the viral entry mechanism.

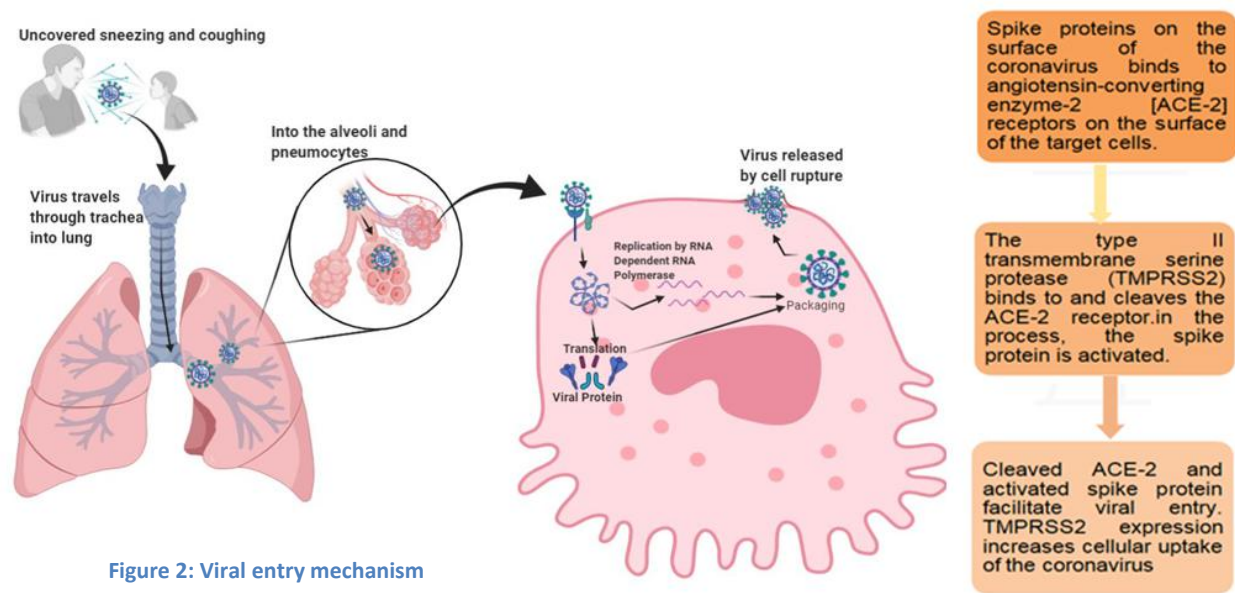


Figure 2: Viral entry mechanism

IV. ANTIGEN PRESENTATION DURING HUMAN SARS COV2 INFECTION

Antigen presenting cells (APC) are mainly involved in presenting viral antigenic peptides to immune cells by MHC I and MHC2 to T cells. The antigen presentation process of SARS-CoV-2 is not yet well understood. Dendritic cells play a crucial role as APC, and is known to be a potential candidate for antigen presentation during SARS infection [14]. The SARS-CoV-2 proteome is known to have been successfully sampled and was represented by a diversity of HLA alleles. However, it was found that HLA-B*46:01 had the fewest predicted binding peptides for SARS-CoV-2, suggesting that individuals with this allele may be particularly vulnerable to COVID-19, as they were previously shown to be for SARS [15]. Further, researchers also found that HLAB*15:03 showed the greatest capacity to present highly conserved SARS-CoV-2 peptides that are shared among common human coronaviruses. In an experiment performed by Bortolotti et al., it was found that a **8mer peptide** in the SP1 domain showed a high affinity mainly for the HLA-E*0101 binding groove and in a lower extent for HLA-E*0301 allele with a similar consensus motif with HLA-E binding [16]. **Table.1** gives a synopsis of the various immunologic changes that have been observed and documented in case of COVID-19 patients.

Table 1: Summary of Immunologic changes that has been documented till date regarding SARS-COV2

Immunologic changes	COVID-19
T-cell responses	Lymphopenia in severe cases (<20%). Initial lymphopenia is predictive of severe disease.
CD8⁺ T cells	Severe lymphopenia (<5%) is observed in case of the lymphocyte subset CD8⁺T_{Cytotoxic} cells and can be a predictor of severe disease.
T_{Helper}1- T_{Cytotoxic} responses	Normal antiviral immunity requires response from the two lymphocyte subsets- CD4⁺T_{Helper} 1 cells and CD8⁺T_{Cytotoxic} cells. Severe disease shows a systemic severe inflammatory response with a cytokine storm. Cytokine storm response is mainly T_{Helper} 1 cell mediated and is inflammatory.
Eosinophils	Decreased circulating eosinophil numbers in 50%-80% of the hospitalized patients.
Specific antibody levels	In the acute phase, virus-specific Immunoglobulin M increases followed by virus-specific Immunoglobulin G during recovery.
Cytokine storm	Innate and adaptive cytokines are released in high amounts linked to severe disease.
Acute-phase reactants	High in severe cases. Initially high values are predictive of severe disease.

V. NATURAL KILLER CELLS

These are large granular lymphocytes, constituting 5-10% of all lymphocytes in human peripheral blood are described phenotypically as CD3⁻ CD14⁻ CD19⁻ CD56⁺ CD16^{+/-} (17). It is associated with cytotoxic activity against tumors and some virus infected cells. In terms of protection, it is the first line of defense against viral infection. Upon viral infection, host cells become more susceptible to NK cell killing through:

- (i) **upregulation of self-encoded molecules induced by infection/cellular stress**(18) that bind activating NK cell receptors such as Natural Cytotoxicity Receptors (NCRs) (NKp30, NKp44, and NKp46) (19), C-type lectin-like receptors NKG2D and NKp80 (20), and co-activating receptors such as DNAM-1 (21);
- (ii) **downregulation of ligands for inhibitory receptors such** as Killer Immunoglobulin-like Receptors (KIRs) (22) and **the C-type lectin-like receptor CD94-NKG2A**(23) which suppress NK cell activation, and;
- (iii) **direct recognition of viral moieties**, via engagement of PAMPS (24) or transmembrane activating receptors such as mouse Ly49H (25) or human NKG2C (26). Moreover, NK cells can eliminate virus-infected cells via CD16-mediated antibody-dependent cell-mediated cytotoxicity (ADCC), which has been shown to be particularly important for herpesvirus clearance (27).

Distinguishing an infected cell is done by joint action of activating receptors and inhibitory receptors. If there are no inhibitory signal, by inhibitory receptor, then the target cell is destroyed. CD94/NKG2A on interacting with HLA-E on a cell marks it to be healthy(28). There are activating receptors like CD94/NKG2C, CD16 that allows killing of target cell(28). Cytokine receptors for IL-1, IL-2, IL-12, IL-15, IL-18, IL-21 are present. Several chemotactic receptors present like -CCR-2, CCR-5, CCR-7, CXCR-1, CXCR-3, CXCR-4, CXCR-6. Off note, **the C-type lectin-like receptor CD94-NKG2A** which suppress NK cell activation has gained prominence as various labs all over the world are working with this receptor with an aim in manipulating it in favor of the host. NKG2A is a heterodimeric inhibitory receptor expressed prominently by cytotoxic lymphocytes, such as NK cells and CD8⁺ T cells, that are thereby endowed with the ability of sensing the level of "self" MHC class-I on target cells. This receptor binds a nonclassical minimally polymorphic HLA class I molecule (HLA-E), which presents peptide domains derived from leader peptide sequences of other HLA class I molecules, such as HLA-G (28). Upon binding with peptide-loaded HLA-E, NKG2A transduces inhibitory signals through two inhibitory immune-receptor tyrosine-based inhibition motifs, thus suppressing the cytotoxic activity of these immune cells, and promoting viral spreading during a variety chronic viral infections (e.g., polyoma virus or human cytomegalovirus)

Table.2 depicts the Main surface markers of the lung NK cells which have become all the more important with reference to SARS-COV-2

Table 2: Depicts surface markers of Lung NK cell which have become important with reference to SARS-COV2

Relevance and function	NK cell-surface molecules
Activating receptors	NKG2D, DNAM1 ^h , NKp30 ^h , NKp44 ^h , NKp46, NKp80 ^h , CD16
Inhibitory receptors	CD94/NKG2A, ILT2 ^h , KIR2DL ^h , KIR2DL2 ^h
Activation marker	CD69
Mature and differentiation markers	KIR ^h , CD57 ^h , CD11b, CD43, CD49b, CD122, Ly49s ^m
Tissue-resident markers	CD49a, CD69, CD103
Adhesion molecules	CD11b, CD49a, CD49b, CD57 ^h , CD103,

^hExpressed only by human NK cells.

^mExpressed only by mouse NK cells.

The NK cell activity is also modulated by a repertoire of cytokines, including, but not limited to, the activating cytokines interleukin (IL)-2/12/15/18 (29) and Type I Interferon (IFN), which can be produced by virally infected cells or activated antigen presenting cells (30, 31). IL-2/12/15/18, alone or in combination, promotes NK cell survival, proliferation, cytotoxicity, and cytokine production, including IFN- γ (32). **Figure.3** gives a schematic over view of the immune activation cascade by NK cells. Macrophages produce IL-12 and help in recruitment and activation of T_H1 cells which secrete IFN- γ , a signature cytokine that activates macrophages and DCs and thereby enhances their ability to kill intracellular microbes and to present antigens to T lymphocytes. T_H1 cells can also secrete tumor necrosis factor (TNF), lymphotoxin(LT), and IL-2, which contribute to antimicrobial defense as well. IL2 plays a dual role in activation and proliferation of T cells and maintenance of T-regulatory(Treg) cells.

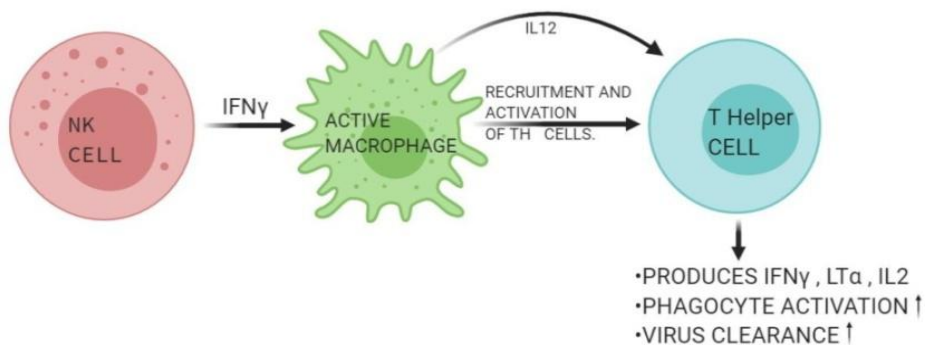


Figure 3: Activation of T lymphocyte by NK Cell- Macrophage signalling

VI. SARS COV2 AND NK CELLS – FUTURE PERSPECTIVE & THERAPEUTIC APPROACH

It has been observed that SARS Cov2 spike 1 protein peptide can be presented by HLA E to NK Cells for immune evasion. Interaction of HLA E with CD94/ NKG2A on NK cells sends inhibitory signals, altering its cytotoxic activity. Thus NK cells leave those infected cells unharmed, and also no further cross talk with adaptive immunity occurs via *macrophage –Th1 pathway* as depicted in **Fig.4**. This in a way might allow the further spread of virus in absence of NK cell action. A significant strategy to tackle the virus in hyper-activated inflammatory COVID-19 patient may be possible if NK cells are activated. The CD94/NKG2A receptor [C type lectin receptor that recognize carbohydrate moiety] can be used as a plausible target for carbohydrate moieties (of the virus), thereby preventing interaction with HLA-E and thus switching off inhibitory signals. Nkp44 receptor is also involved in causing cellular cytotoxicity. In other viral infections it is seen to bind to viral antigen and detect infected cell to kill it. Further investigation is needed to identify such antigen of SARS-Cov2 that may drive the NK cell to its action. Cytokines like IL-6, IL-10 has been seen to increase NKG2A receptor expression on NK cell (33). Targeted inhibition of those cytokine may reduce inhibitory receptor expression and allow proper activation of NK cell against SARS-Cov-2 infected cell.

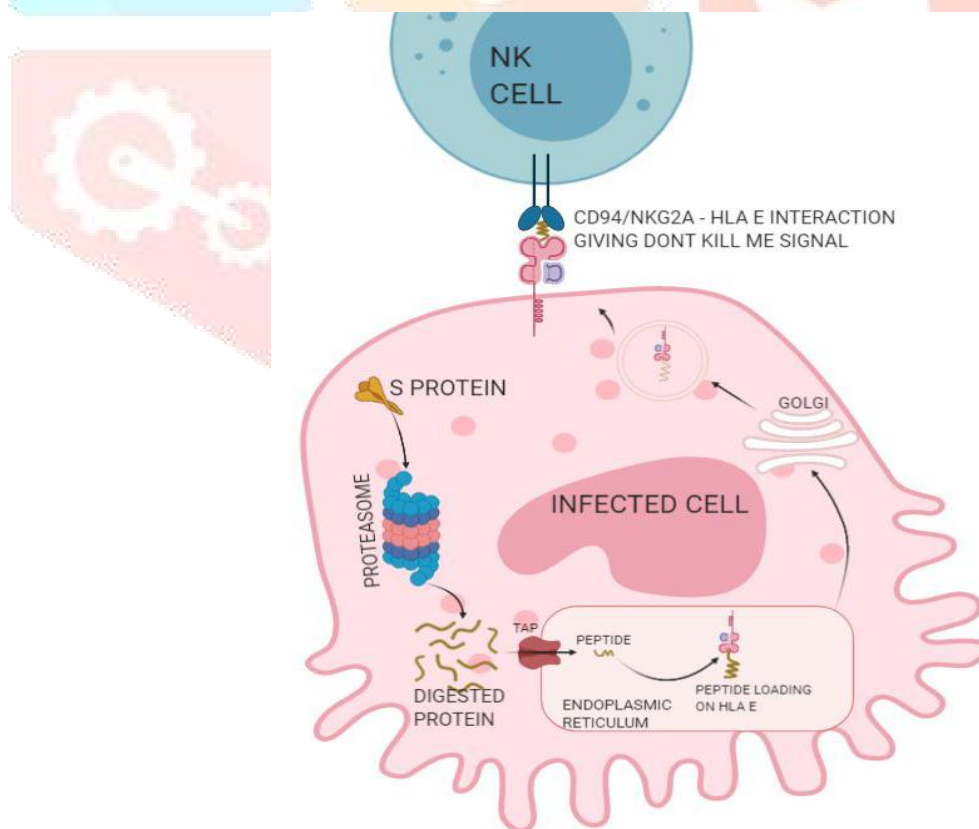


Figure 4: NK cell evasion

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