Double-bright (CD56bright/CD16bright) natural killer cell adoptive immunotherapy for SARS-CoV-2

SARS-CoV-2, affecting predominantly elderlies, was recognized as a global pandemic on 11 March, 2020; clinical signs and laboratory alterations are similar to previous coronavirus epidemics with lymphocytopenia and neutrophilia observed in severe cases. These findings were described in humans in the present pandemic[1] and result in progressive hyperactivation of inflammatory cells (macrophages and neutrophils) leading to SARS and a cytokine storm in its final stages. Additionally, there are coagulopathies believed to be secondary to endothelial-cell infection as virus elements within endothelial cells have been clearly demonstrated.[2] In severe patients there are fewer circulating B, T and natural killer (NK) cells, endorsing the hypothesis that immune dysregulation plays a role in disease severity.[1]

Pulmonary physiology is characterized and regulated by a unique mixture of alveolar epithelial cells (AEC Type I and Type II) and resident immune cells. Type I AECs line alveolar spaces, and through their thin cytoplasm, efficient gas exchange is accomplished; in addition to producing surfactant, Type II AECs regulate Type I regeneration and homeostasis. Inflammation interferes dangerously with gas exchange efficacy. Resident alveolar macrophages (rAM) appear to be central in maintaining pulmonary homeostasis through their anti-inflammatory phenotype, actively suppressing the local cellular immune response (M2 phenotype).[3] New respiratory pathogens activate rAM that acquire an inflammatory phenotype (M1) and activate resident dendritic cells (rDC). Pulmonary resident NK cells (rNK) with a peculiar quiescent phenotype, once activated, can also cross-talk with rDC which upon activation migrate to the lung, draining lymph nodes to initiate T and B cell response.[4,5]

Ageing has an adverse impact in pulmonary immunology, and its impact on NK cell function results in an increased susceptibility to tuberculosis re-activation, fungal and bacterial infections secondary to a decrease both in IFN-gamma production and expression of natural activating receptors such as NKp30 and NKp46; NK cell immune senescence contributes to the higher incidence of viral infections as well.[6]

NK cells are innate large granular lymphocytes capable of lysing altered cells without previous exposition; their hallmark is the presence of killer immunoglobulin-like receptors (KIRs) able to either inhibit or activate NK cell activity. One's own normal cells are spared from NK cell lysis since KIR inhibitory receptors sense self-class I HLA antigens. Classically regarded as only cytotoxic, the role of NK cells in the activation of an adaptive immune response has been increasingly recognized. NK cell function goes beyond the recognition and elimination of transformed, viral-infected or antibody-coated cells with a role in antimicrobial infection, clearance of senescent cells and resolution of inflammation. The latter is of particular interest since NK cell neutrophil apoptosis attenuates local tissue damage.[6]

NK cell receptors' genotype and their ligands modulate AIDS development in human immunodeficiency virus (HIV)-positive individuals; at early stages of infection NK cells appear to be involved in controlling virus replication rate, and HIV's immune escape relays on the virus's ability to promote disturbances in NK cell homeostasis that is restored with effective antiretroviral therapy.[7] In infants with respiratory syncytial virus (RSV), NK cell-mediated antibody-dependent cellular cytotoxicity (ADCC) was shown to protect against severe disease.[8] In patients with hepatitis C virus (HCV), activated NK cell numbers in hepatic tissue are inversely correlated with the levels of viral RNA and degree of fibrosis.[9] Specific anti-HCV, -hepatitis B virus (HBV), -cytomegalovirus (CMV), -influenza virus, -hantavirus and -HIV memory NK cells were also described.[10] Early NK cell antiviral activity in the resolution or clinical course severity attenuation of flavivirus infections such as West Nile virus (WNV), yellow fever (YFV), dengue (DENV) and Zika (ZIKV) or Chikungunya (CHIKV) alphavirus have been well documented. In these diseases, severe clinical manifestations were related to either NK cell dysfunction or its genetic characteristics utilized by the virus to overcome NK cell activity.[11] Finally, genetic NK cell deficiency is characterized by severe and disseminated varicella zoster, CMV, Epstein–Barr or herpes simplex viral infections.[12]

SARS-CoV-2-infected patients have a decreased number of circulating NK cells,[1] and in the predominant immune-suppressive lung microenvironment, senescent dysfunctional NK cells probably are not properly activated.[6]

In cancer, particularly in myeloid leukaemias, the adoptive transfer of *ex-vivo*-selected and activated NK cells is safe and displays anti-leukaemia activity.[13]

We utilize an *ex vivo* expansion platform based on genetically modified feeder cells expressing membrane-bound interleukin (IL)-21 (mbIL21) that gives rise to highly activated, clinical-grade Double-Bright (DB; $CD56^{bright}$ and $CD16^{bright}$) NK cells. In a recent interim analysis of a phase I/II clinical trial on DB-NK cells for relapsed/refractory acute myeloid

leukaemia (R/R AML) results were encouraging: 78·6% of the overall response rate (ORR) (manuscript in preparation) without infusion-related toxicity. In this study, we saw unexpected favourable activity in subgroups of patients with central nervous system (CNS) leukaemia and with severe infection; in two of the latter, unsuspected pulmonary tuberculosis and aspergillosis were diagnosed as a NK cell infusion-emergent effect, with no hypoxia, and symptoms subsiding days after emergence.[14] In fact, none of our 14 DB-NK cell adoptive immunotherapies caused hypoxia, neither during DB-NK cell infusions nor later in their clinical course.

RNA analysis of expanded DB-NK cells utilizing our manufactory platform revealed the absence of ACE-2 receptor (Dean A. Lee, personal communication).

Lessons learned from the passive transfer of antibodies with convalescent plasma to treat infectious diseases indicate better results when the transfer is done earlier in the course of the disease. Convalescent plasma appears effective for SAR-CoV-2; however, it is becoming clear that severe patients already in the overwhelming cytokine storm and coagulopathy phase are less prone to have a benefit.[15]

Since the idea is to establish an adaptive immune response as early as possible, and to that end, to attempt to overcome lung immune suppression by infusing highly activated DB-NK cells, it is our belief that NK cells should be infused early in the course of the disease since time to develop a robust immune adaptive response is the key point of the proposed therapy.

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Transfusion demand in COVID-19 patients from the Korean population: a nationwide study in South Korea

As patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) often present coagulopathy depending on disease severity, appropriate prevention and treatment for haemodynamics control are most essential for coronavirus disease 2019 (COVID-19) management.[1] Although several papers warned that, as expected, there was a decrease in the number of blood donations in the COVID-19 pandemic,[2,3] few studies investigated the actual prevalence of blood transfusions requested in hospitalized COVID-19 patients,[4,5] and no publication has reported nationwide