Focusing On A Unique Innate Memory Cell Population Of Natural Killer Cells In The Fight Against COVID-19: Harnessing The Ubiquity Of Cytomegalovirus Exposure

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Introduction. The current pandemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) manifesting as severe pneumonia in a subgroup of patients has generated interest in the immunological response to this virus or the lack of it. Despite being a novel virus, many of the patients are either asymptomatic or mildly symptomatic. On the other hand, a subgroup of patients demonstrates a severe cytokine storm, similar to that witnessed in the macrophage activation syndrome (MAS) or hemophagocytic syndrome (HPS). This response begs the question if there exists innate protection against this new pathogen and, if so, what that might be.

NKG2C+ Natural Killer Cells: Innate Memory Cells and Cytomegalovirus. Natural Killer (NK) cells are the frontline warriors in the launch of antiviral immunity. However, these cells act in an antigen-independent manner and last for a short while, a characteristic of the innate immune response. In mice, it was observed that NK cells bearing Ly49H receptors were preferentially expanded in response to cytomegalovirus (CMV) infection, the viral protein m157 being the trigger. More importantly, these cells persisted and expanded in response to re-challenge with cytomegalovirus (CMV) infection, the viral protein m157 being the trigger. More importantly, these cells persisted and expanded in response to re-challenge with the virus, mimicking a memory response akin to the adaptive immune counterpart. A similar subtype was identified in humans with the expansion of NK cells expressing a C lectin type receptor NKG2C, when cultured with CMV infected fibroblasts. This subtype was further characterized by the absence of the corresponding inhibitory receptor NKG2A, a high expression of CD57 receptor (a marker of maturation), an amplified antibody-dependent cellular cytotoxicity through the CD16 receptor and a strong interferon-gamma (IFNγ) response. While conventional effector NK cells are short-lasting, these cells were found to last for years in CMV seropositive individuals, but not in those who are CMV naïve. Thus, the NKG2C subset of NK cells was identified as a unique subgroup of innate cells with a memory phenotype generated by CMV infection. However, these cells were found to expand in response against several RNA viruses as well.

Hypothesis. Our group has been working on this memory subset of NK cells following haploidentical hematopoietic cell transplantation (HCT) with a novel approach of T cell costimulation blockade with CTLA4Ig. High NKG2C+ NK cell levels were observed to be protective against the recurrence of CMV reactivation, as well as reactivation of adenovirus in this protocol. In addition, NKG2C+ NK cells inversely correlated with graft-versus-host disease (GVHD) following haploidentical HCT in our study, confirming similar findings reported by others as well. This has led us to speculate whether these cells may be protective against the SARS-CoV-2 infection as well. If so, the possibility of these cells being isolated and expanded from haploidentical family donors and used for adoptive immunotherapy against COVID-19 is worth consideration. The following sections discuss the rationale for this proposition and its possible implications.

NKG2C+ NK Cells In Other Viral Infections. This subset of NK cells with NKG2C expression has also been found to rapidly expand following influenza vaccination with increased IFNγ release. Prolonged memory response to influenza strains is uncommon and annual vaccination is recommended. In a subgroup of 21 HCT recipients prospectively evaluated by us during a seasonal outbreak in 2019, 8 developed H1N1 infection. All eight patients had high NKG2C+ NK cell levels (22.1 ± 9.5%), with a substantial increase in these cells four weeks following the infection (41.8 ±
13.3%). All patients had mild to moderate symptoms with none developing lower respiratory infections, suggesting a possible role of expanded NKG2C+ NK cells in retarding the progression of H1N1 infection. A similar expansion of NKG2C+ NK cells has been reported following Hantavirus infections, who were CMV seropositive. This subset of NK cells was also associated with a lower viral load in those with HIV infections. These observations suggest that while CMV infection is essential for the development of the NKG2C subset of the NK cell population, this population may also functionally expand in response to a wide array of viruses. The response against both DNA and RNA viruses in an antigen-independent manner, in terms of further expansion, sets these NK cells apart from memory cells of adaptive lineage, which are highly antigen-specific in their response. A similar memory response of NK cells has been demonstrated against mycobacterium in mouse models. In patients with latent infection with mycobacterium tuberculosis, vaccination with Bacillus Calmette–Guérin (BCG) induced a long term NK cell response lasting for 12 months.

Can NKG2C+ NK Cells Provide Protective Immunity Against COVID-19? Studies on immunological predispositions and their consequences, concerning coronavirus disease 2019 (COVID-19), are still scant. However, a study from China has shown an increase in the expression of NKG2A receptors in NK cells following SARS-CoV-2 infection and a corresponding decrease in this inhibitory receptor following recovery. Even though the report does not allude to the NKG2C+ NK cell subset, it is worth noting that the expression of NKG2A inversely correlated with NKG2C, as shown by our group and also by others. HLA E is a ligand for both NKG2A as well as NKG2C receptors, with the former binding its ligand with a higher affinity. Thus, the absence of NKG2A expression is a pre-requisite for the binding of NKG2C to the putative receptors and further cytotoxicity. Virus infections often upregulate HLA-E expression on the infected cells, preventing its killing by NK cells expressing NKG2A, despite having the full repertoire of cytotoxic receptors. Thus, a higher proportion of NKG2A-NKG2C+ NK cells might offer better protection against such infections. In addition, the correlation between high expression of NKG2A and an adverse outcome in those with COVID-19 has prompted researchers to speculate if monoclonal antibodies against NKG2A such as Monalizumab may potentially reverse the inhibition of the innate immune system induced by the virus.

Furthermore, in-vitro studies have shown that signaling via specific Notch ligands in cord progenitor cells, such as Jagged2, Delta1, or Delta4, may result in the expansion of an immature phenotype of NK cells without NKG2A expression. These NKG2A-KIR-CD56bright NK cells were shown to upregulate receptors such as NKp44, NKp30, and DNAM-1. This does not conform to the natural stages of maturation of NK cells in-vivo and rather reflects an accelerated functional ability despite an immature phenotype, characterized by augmented release of IFNγ and cytotoxicity against the K562 cell line- a functional phenotype resembling NKG2A-NKG2C+ NK cells. If developed clinically, this might represent a novel way of harnessing functional NK cells from cord blood, enabling the genesis of an off the shelf product for antiviral as well as anticancer immunotherapy.

Harnessing Antigen-Independent Properties of Innate Memory Cells in COVID-19. In the earlier widespread coronavirus infections as in SARS-CoV-1, a lack of cytotoxic NK cells was strongly correlated with the severity of the disease. This has drawn attention to the possible use of NK cell-based immunotherapy in COVID-19. Derived from the above findings, we hypothesize that this unique group of innate immune cells may have a specific protective role in COVID-19. Given the large number of patients infected with this virus across the globe, a study of the role of NKG2C+ NK cells becomes both pertinent and urgent, as these cells may be boosted by influenza vaccination as well as BCG and can be generated ex-vivo for adoptive immunotherapy from CMV seropositive donors, as prior CMV infection is a prerequisite for the generation and expansion of this unique population of NK cells with a memory phenotype. Furthermore, the fatal HPS often found in fatal cases of COVID-19, is an end-result of abortive NK cell cytotoxicity, lending further credence to the hypothesis that cytotoxic NK cells and the NKG2C subset, in particular, may protect against severe disease in patients infected with COVID-19. Our early results on the use of heat-killed Mycobacterium w (Mw) in patients diagnosed with Covid-19 infection are encouraging.

If that be the case, an early immunological intervention might reduce disease severity as well as mortality. We have proposed a similar study to evaluate the NK cell subsets in those exposed to SARS-CoV2 and those developing the disease in India as CMV seropositivity is ubiquitous in the population. The fact that NKG2A-NKG2C+ NK cells have been found to be protective against GVHD in haploidentical HCT, despite having a potent antiviral and antitumor activity, is encouraging. If found to be protective, this may indicate an opportunity to intervene and boost this subset through either vaccination or adoptive immunotherapy from family donors.
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