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Human Adaptive Natural Killer Cells: Beyond NKG2C

Julia A. Wagner¹ and Todd A. Fehniger^{1,*}

¹Department of Medicine, Division of Oncology, Washington University School of Medicine

Paradigm-shifting studies have identified NKG2C⁺ adaptive natural killer (NK) cells in individuals infected with cytomegalovirus. Recently in *Cell Reports*, Liu *et al.* demonstrate that NKG2C^{−/−} HCMV⁺ individuals also generate adaptive NK cells, and reveal CD2 as a major co-stimulatory receptor for these NK cells specialized to respond via FcγRIIIa/CD16.

The past decade has borne witness to an explosion of studies identifying memory or memory-like properties in classically defined innate immune cells. Natural killer (NK) cells are no exception, with several types of innate memory-like or adaptive responses described in mice following exposure to haptens, viral infection (i.e., murine cytomegalovirus, MCMV), or combined IL-12, IL-15, and IL-18 cytokine activation [1]. The unifying concepts behind these different types of innate memory are that naive NK cells are i) exposed to a stimulating environment, ii) removed from the initial stimulus and returned to a baseline activation state, and then iii) more robustly responsive when specifically (in the case of haptens) or non-specifically (in the case of viral infection and combined cytokine pre-activation) re-stimulated. These studies prompted a search for human NK cell innate memory or memory-like responses. Recent studies have identified adaptive features of human NK cells following human cytomegalovirus (HCMV) infection or re-activation, as well as following IL-12, IL-15, and IL-18 combined activation [2].

NKG2C is a C-type lectin activating receptor expressed as a heterodimer with CD94 that recognizes the non-classical MHC class I molecule HLA-E. While human NK cells express NKG2C to varying degrees, numerous studies have now established that populations of NKG2C⁺ NK cells are present at higher frequencies within the NK cell compartments of individuals seropositive for HCMV [3]. An increased frequency of NKG2C⁺ NK cells was also observed in the setting of CMV reactivation in patients following solid organ and hematopoietic cell transplantation, which correlated with HCMV serostatus [4,5]. Moreover, NKG2C⁺ NK cells expanded within HCMV seropositive hematopoietic cell transplant recipients, and produced greater levels of IFN-γ, particularly when CMV antigens were present [6]. These observations led to the general acceptance that the human NK cell compartment adapts over time to HCMV infection, with the end result being an increased number of NKG2C⁺ NK cells with specialized properties. These attributes include increased expression of NKG2C, CD2, and CD57, but reduced expression of the activating receptors

*Correspondence: ; Email: tfehnige@wustl.edu (T.A. Fehniger)

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NKp46, NKp30, and CD161. More recent reports have identified a key role for FcγRIIIa (CD16) binding to antibody-opsonized HCMV-infected cells in this adaptive NK cell response and expansion [3]. Thus, adaptation of the NK cell compartment may result from long-term NK cell activation via FcγRIIIa binding to anti-HCMV antibodies present in HCMV-seropositive individuals. Further, this functional enhancement has now been mechanistically explained by alterations in multiple intracellular proteins, including reduced expression of signaling adapter proteins (FcεR1γ, Syk, Eat-2, Dab-2) and transcription factors (PZLF and Helios). Several of these findings were linked to changes in DNA methylation, supporting the idea that adaptive NKG2C⁺ NK cells represent a specialized NK cell type or differentiation state [3]. It should be noted that while most HCMV-seropositive individuals have identifiable NKG2C⁺ adaptive NK cell populations, the fraction of NKG2C⁺ NK cells can vary greatly (i.e. from 2% to >50%) between HCMV seropositive people. The reasons underlying this extensive heterogeneity and its implications for health and disease remain largely unknown.

Functional experiments have also identified that adaptive NK cells preferentially produce IFN-γ and other cytokines in response to FcγRIIIa triggering. This observation was initially explained by selective use of the more ‘potent’ (additional ITAM motif-bearing) activating adapter CD3ζ, which is preferentially utilized when FcεR1γ expression is reduced [3]. However, reports of FcγRIIIa-triggered degranulation (inferred via CD107a cell surface expression) were not different from conventional NK cells, while other functions such as natural killing were reduced in adaptive NK cells. NKG2C appeared to mark adaptive NK cells, but questions remained about the role and requirement for NKG2C in adaptive NK cell populations. Is NKG2C engagement required for adaptive NK cells to differentiate? What is the role of NKG2C in adaptive NK cell FcγRIIIa-triggered IFN-γ responses?

In a recent article in *Cell Reports*, Liu *et al.* address these questions by studying 60 NKG2C-deficient individuals identified from a screen of over 2200 individuals [7]. Adaptive NK cells were found to be present to an equivalent extent in NKG2C⁺ and NKG2C^{-/-} individuals infected with HCMV, which is consistent with a prior report that adaptive-type NK cells were generated following CMV reactivation in 3 recipients of NKG2C^{-/-} umbilical cord blood transplants [8]. The adaptive NK cells of NKG2C⁺ and NKG2C^{-/-} individuals shared numerous functional and phenotypic attributes including preferential expression of certain KIRs, minimal cytokine receptor-based activation, decreased responses upon direct exposure to conventional tumor targets, enhanced FcγRIIIa-triggered IFN-γ and TNF production, and demethylation of the *ifng* locus CNS1 region (Figure 1). Importantly, the authors identified CD2 as a co-stimulatory molecule for antibody-dependent adaptive NK cell functional responses in both NKG2C^{-/-} and NKG2C⁺ individuals. Only CD2 engagement synergized with FcγRIIIa-triggered functional responses in adaptive NK cells. Consistent with this functional synergy, CD2 co-stimulation led to a broad enhancement of signaling molecule phosphorylation downstream of FcγRIIIa in adaptive human NK cells. Thus, while NKG2C to HLA-E interactions may promote adaptive NK cell generation [3], this receptor interaction does not appear to be absolutely required. Since NKG2C⁺ expansions have also been observed in response to some viral infections (e.g. HCMV, Hantavirus, and Chikungunya) but not others (e.g., EBV), it will be of interest to evaluate

patients chronically infected with viruses for defined, and potentially novel, adaptive NK cell signatures.

Collectively, these findings shed light on human NK cell functional specialization in response to a latent herpesvirus, and raise important questions about the role of such adaptive NK cells in the host response to viruses and tumor cells. As an NK cell subset that is focused on responding to antibody-opsonized target cells and producing effector cytokines, adaptive NK cells may be specialized to respond to continuous sub-clinical CMV reactivations via HCMV-specific (and potentially other) antibodies. In contrast, adaptive NK cells respond weakly to tumor target cells and cytokine receptor-based activation compared to conventional NK cells, potentially limiting their responses to tumors and at sites of inflammation. Adaptive NK cell populations have been shown to be present in HCMV seropositive individuals in the setting of hematologic malignancy [9], which could diminish non-FcγRIIIa-triggered NK cell responses in cancer patients. In contrast, the use of therapeutic anti-tumor monoclonal antibodies may be influenced by the presence and relative abundance of adaptive NK cells in cancer patients. Directly related to the findings of Liu *et al.* [7], it will be of interest to define the extent to which CD2 co-stimulation is required for *in vivo* anti-tumor or anti-viral responses, and thus whether adaptive NK cells are restricted to targeting CD58-expressing cells. It will also be informative to investigate whether the potential for CD2 co-stimulation is a property of other types of memory-like NK cell responses in mice and humans.

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References

1. Min-Oo G, et al. Natural killer cells: walking three paths down memory lane. *Trends Immunol.* 2013; 34:251–258. [PubMed: 23499559]
2. Berrien-Elliott MM, et al. Human cytokine-induced memory-like natural killer cells. *J Innate Immun.* 2015; 30:563–571. [PubMed: 25924651]
3. Rölle A, Brodin P. Immune adaptation to environmental influence: the case of NK Cells and HCMV. *Trends Immunol.* 2016; 37:233–243. [PubMed: 26869205]
4. Lopez-Verges S, et al. Expansion of a unique CD57+NKG2C high natural killer cell subset during acute human cytomegalovirus infection. *Proc Natl Acad Sci USA.* 2011; 108:14725–14732. [PubMed: 21825173]
5. Foley B, et al. Cytomegalovirus reactivation after allogeneic transplantation promotes a lasting increase in educated NKG2C+ natural killer cells with potent function. *Blood.* 2011; 119:2665–2674. [PubMed: 22180440]
6. Foley B, et al. Human cytomegalovirus (CMV)-induced memory-like NKG2C(+) NK cells are transplantable and expand *in vivo* in response to recipient CMV antigen. *J Immunol.* 2012; 189:5082–6008. [PubMed: 23077239]
7. Liu LL, et al. Critical role of CD2 co-stimulation in adaptive NK cell responses revealed in NKG2C-deficient humans. *Cell Rep.* 2016; 15:1–12. [PubMed: 27052168]
8. Della Chiesa M, et al. Human cytomegalovirus infection promotes rapid maturation of NK cells expressing activating killer Ig-like receptor in patients transplanted with NKG2C^{−/−} umbilical cord blood. *J Immunol.* 2014; 192:1471–1479. [PubMed: 24442432]

9. Petersen L, et al. The CD94/NKG2C-expressing NK cell subset is augmented in chronic lymphocytic leukemia patients with positive human cytomegalovirus serostatus. *Viral Immunol.* 2009; 22:333–337. [PubMed: 19811091]

Figure 1. HCMV Adaptive Human NK Cells are Present in NKG2C^{-/-} Individuals and Utilize CD2 as a Primary Co-Stimulatory Receptor

A study of NK cells within rare NKG2C^{-/-} HCMV⁺ individuals revealed a profile of cell surface markers and intracellular protein expression characteristic of adaptive NK cells. These cells were directly related to previously reported NKG2C⁺ adaptive NK cells from NKG2C^{+/+} individuals. Thus, HCMV adaptive NK cells do not *a priori* require NKG2C to develop within HCMV seropositive individuals. In addition, CD2 was identified as a major co-stimulatory receptor acting in concert with FcγRIIIa to stimulate increased IFN-γ and TNF production upon antibody recognition. HCMV adaptive NK cells have the following properties: increased surface expression of CD57, LILRB1, and NKG2C when present; decreased surface expression of CD7, NKp46, NKp30, CD161, NKG2A; decreased intracellular expression of FcεR1γ, Syk, EAT-2, PLZF, Helios, DAB2; and decreased *ifng* gene methylation.