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Comment on White et al, page 4377

Latent herpesviruses: aligning human and murine NK cells

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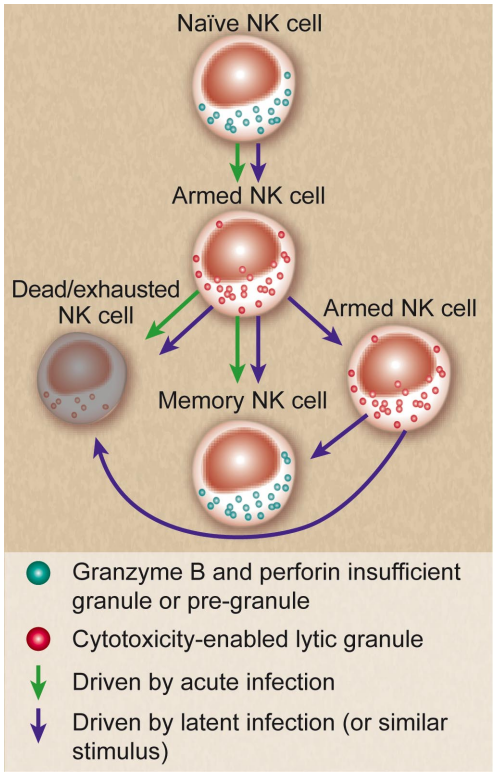
In this issue of *Blood*, White and colleagues demonstrate that naive murine NK cells can be armed as cytolytic effectors by latent herpesvirus infection, thus suggesting a mechanism by which herpesviruses may routinely make NK-cell functions accessible to the host.



Role for latent herpesvirus infection in NK-cell arming. The data presented by White et al suggest a model in which naive NK cells in mice can be induced to become armed lytic effectors by latent infection with MuHV-4. Murine NK cells typically exist under pathogen free conditions in an unarmed state without high-level expression of granzyme B and perforin. While it is unclear in what state of maturity lytic granules may be formed in unarmed NK cells, granzyme A is expressed, but morphologic assessments define a lack of fully matured granules.[3] Presumably, after an acute infection (green arrows), an armed NK cell will participate in killing and either end its lifecycle via death or revert to a less armed state but persist as a memory NK cell. These latter cells do not appear armed, but retain potential to more actively participate in immune responses.[12,13] The data suggest that unlike acute infection, latent infection is capable of not only arming NK cells, but maintaining them in an armed state (purple arrows). Since human NK cells are typically armed at baseline, this raises a role for latent infection or similar signals in enabling their continually armed state. While the mechanism of how latent infection promotes arming is unclear, the majority of armed cells in a latently infected host would not be fighting active infection and degranulating. Therefore, in the case of latent infection, it would be predicted that many will continue to persist in an armed state, and not simply progressing to death or memory-like phenotypes. Without latent infection or similar signals derived from other environmental challenges (such as in SPF mice), the majority of NK cells will exist in the naive state. This is therefore not seen in humans, as environmental challenges and latent herpesviral infections are ubiquitous. Professional illustration by Paulette Dennis.

Natural killer (NK) cells are the major cytolytic lymphocytes of the innate immune system and can be induced to kill target cells or produce cytokines after the ligation of activation receptors encoded in the germline DNA.[1] To mediate cytotoxicity, NK cells mobilize lytic granules containing perforin and granzymes toward a target cell and secrete their contents in a directed manner.[2] The vast majority of human NK cells exist in an "armed" (or primed) state containing abundant lytic effector molecules including perforin and granzyme. A major and unexplained difference between human and murine NK cells, however, is that animals maintained under specific pathogen-free (SPF) conditions have low ex vivo NK-cell function. This was previously defined by the senior author of the present study as a feature of reduced granzyme B and perforin protein, despite there being fully developed NK cells expressing abundant levels of granzyme B and perforin mRNA.[3] While humans do not exist under SPF conditions, what is it about the human condition that allows for human NK cells to be armed at baseline? It could be hypothesized that tonic stimulation from the microbial environment is sufficient and results in adequate signals to continually drive human NK-cell arming. In this light, several cytokine-related signals have been previously demonstrated to enable the priming of murine NK cells.[3,4] However, White et al define a specific enabler for NK-cell arming that may have direct relevance to the human condition: latent herpesvirus infection.[5]

The focus on latent herpesviruses was born out of earlier observations that more robust immunity has been defined in mice latently infected with herpesviruses.[6] The concept is also practically appealing, as herpesviruses are fairly ubiquitous in humans and evidence of herpesvirus genomes is frequently found in otherwise healthy persons who have never demonstrated infection.[7] Furthermore, the direct involvement of NK cells in control of herpesviral infection is well documented in humans and other species.[8,9]

The authors of the present study use a γ-herpesvirus, murid herpesvirus 4 (MuHV-4), and evaluate mice during acute infection when virus is actively replicating as well as 28 days after infection, when a state of latency has been defined. Here, they show that NK cells become armed during acute infection as would be expected, but remain armed once infection has become latent. During latent infection, armed NK cells become the majority NK-cell population in the spleen, thus more directly mirroring human peripheral blood NK cells where the majority persist in

an armed state. It remains to be defined, however, whether the latent infection is sufficient to enable the indefinite persistence of armed NK cells in the mouse. In the setting of *Listeria* infection, MuHV-4 infection was shown to lead to enhanced immunity for months, but not indefinitely.[10]

White et al demonstrate the arming phenomenon as a feature of latent infection in several ways. First, splenocytes from RAG$^{-/-}$ mice housed under SPF conditions demonstrated increased granzyme B protein expression 72 hours after adoptive transfer into a latently infected recipient animal. This did not require proliferation, as the transferred cells became armed but did not dilute a CFSE label. Second, the authors made use of a mutant MuHV-4 (073.stop virus), which has a genetically defined reduced capacity to establish latency. Interestingly, infection with the 073.stop virus resulted in a typical NK-cell response to acute infection with increased cytotoxicity and granzyme B expression, but failed to lead to the presence of NK cells with increased granzyme B protein after the virus had been cleared. Thus, arming as defined by granzyme B expression and increased capacity for cytotoxicity was a feature of the latent infection. This translated to increased NK-cell function and host defense, as only mice with wild-type MuHV-4 latent infection were able to survive a challenge with RMA-S tumor cells (a classical model of in vivo NK–cell activity).

The mechanism of how latent infection arms NK cells, however, was not explored and remains unclear. Latency of MuHV-4 is established in certain lymphoid tissues, thus perhaps representing a special circumstance with regard to access to the NK-cell compartment. As the authors point out, armed NK cells exist among human cord blood, suggesting that mechanisms other than direct exposure to host cells latently infected with herpesviruses are likely to exist.

The present work raises several additional questions. These include how the phenomenon of arming coexists with that of licensing, or the developmental enabling of cytotoxicity due to the appropriate exposure to regulatory signals.[11] Are the majority of armed NK cells licensed, and have they traditionally developed prior to arming? Similarly, if there is a population of armed NK cells that were not conventionally licensed, are there differences in how these cells persist over time compared with those that are licensed? Furthermore, although the authors have previously defined that arming can be regulated at the level of translation, it remains unclear how this relates to maturation and development of the complex lytic machinery itself.[2] It is likely that some lytic effector components are constitutively available and others induced in the process of arming.

Another question is how armed NK cells that develop in the context of latent infection relate to the recently described memory NK cells.[12,13] Can armed NK cells give rise to memory NK cells? There are at least some distinctions between the two, as the memory NK cells are observed after contraction of the response and demonstrate a reduced ability to kill. Thus, perhaps the armed NK cells represent a population that has never had the opportunity to enter into the memory phase due to a persisting signal for arming (see figure). This would be suggested by the experiments using the 073.stop virus, in which armed NK cells did not persist.

Although the demonstration of arming raises important questions, it does potentially bring into alignment some experimental distinctions between human and murine NK cells. It also suggests a benefit to the host of becoming latently infected with herpesvirus, where latent infection facilitates the persistent readiness of NK cells and potentially promotes NK cell–mediated surveillance against tumors, which would presumably lead to improved human survival.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES

1. Lanier LL. Up on the tightrope: natural killer cell activation and inhibition. *Nat Immunol.* 2008;9(5):495-502.
2. Orange JS. Formation and function of the lytic NK-cell immunological synapse. *Nat Rev Immunol.* 2008;8(9):713-725.
3. Fehniger TA, Cai SF, Cao X, et al. Acquisition of murine NK cell cytotoxicity requires the translation of a pre-existing pool of granzyme B and perforin mRNAs. *Immunity.* 2007;26(6):798-811.
4. Chaix J, Tessmer MS, Hoebe K, et al. Cutting edge: priming of NK cells by IL-18. *J Immunol.* 2008;181(3):1627-1631.
5. White DW, Keppel CR, Schneider SE, et al. Latent herpesvirus infection arms NK cells. *Blood*; 2010;115(22):4377-4383.
6. Barton ES, White DW, Cathelyn JS, et al. Herpesvirus latency confers symbiotic protection from bacterial infection. *Nature.* 2007;447(7142):326-329.
7. Kaufman HE, Azcuy AM, Varnell ED, Sloop GD, Thompson HW, Hill JM. HSV-1 DNA in tears and saliva of normal adults. *Invest Ophthalmol Vis Sci.* 2005;46(1):241-247.
8. Biron CA, Nguyen KB, Pien GC, Cousens LP, Salazar-Mather TP. Natural killer cells in antiviral defense: function and regulation by innate cytokines. *Annu Rev Immunol.* 1999;17:189-220.
9. Orange JS. Human natural killer cell deficiencies and susceptibility to infection. *Microbes Infect.* 2002;4(15):1545-1558.
10. Yager EJ, Szaba FM, Kummer LW, et al. gamma-Herpesvirus-induced protection against bacterial infection is transient. *Viral Immunol.* 2009;22(1):67-72.
11. Kim S, Poursine-Laurent J, Truscott SM, et al. Licensing of natural killer cells by host major histocompatibility complex class I molecules. *Nature.* 2005;436(7051):709-713.
12. Cooper MA, Elliott JM, Keyel PA, Yang L, Carrero JA, Yokoyama WM. Cytokine-induced memory-like natural killer cells. *Proc Natl Acad Sci U S A.* 2009;106(6):1915-1919.
13. Sun JC, Beilke JN, Lanier LL. Adaptive immune features of natural killer cells. *Nature.* 2009;457(7229):557-561.

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Comment on Nakahara et al, page 4384

Cyclophosphamide, DCs, and Tregs

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Certain chemotherapeutics are now known to augment host immunity by acting on DCs. In this issue of *Blood*, Nakahara and colleagues demonstrate a unique pharmacologic activity of CTX to selectively eliminate the lymphoid tissue-resident CD8$^+$ DC subset in mice.[1]

Although chemotherapeutic agents are generally believed to suppress the host immunity, recent studies have unveiled unexpected potentials of some agents to augment the adaptive immune responses against cancer cells (see table). For example, anthracins (eg, doxorubicin and mitoxantrone), but not other DNA-damaging drugs, induce an immunogenic form of cancer cell death characterized by surface expression of calreticulin, which in turn promotes efficient phagocytosis of dying cancer cells by dendritic cells (DCs).[2] Cancer