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Natural Killer Cells: Remembrances of things past

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Abstract

Natural killer (NK) cells exhibit a form of memory, previously considered an exclusive property of adaptive immunity. While protective, NK memory is probably hazier and more fleeting than T cell memory.

The immune system is divided into adaptive and innate components [1]. The adaptive immune response, carried out by antibodies, T cells and B cells, is characterized by virtually unlimited diversity in specificity, and exhibits long-term antigen-specific memory responses when a pathogen is reencountered years later. Innate immunity is carried out by many cell types including granulocytes, macrophages, dendritic cells and natural killer (NK) cells. As innate immunity emerged as a field in recent years, textbooks defined cardinal features distinguishing it from adaptive immunity: innate immunity is characterized by recognition receptors of highly limited diversity, which target predictable features of pathogens and diseased cells, and is not accompanied by specific memory. A recent surprise is that NK cells do, in fact, exhibit a key feature of adaptive immunity: memory [2,3].

NK cells are lymphocytes, like T and B cells, and share many properties with CD8 T cells [4]. They are nevertheless considered components of the innate immune system for several reasons: they exist in animals that cannot produce T and B cells due to defects in enzymes necessary for rearranging antigen receptor genes [5]; their recognition receptors do not undergo somatic diversification and are specific for predictable entities such as ligands displayed on distressed cells or specific viral proteins [6]; they were not thought to confer long-term immunity to infections (memory).

In the adaptive immune system, memory is intimately tied to clonal diversity and clonal selection. For example, the frequency of CD8+ (killer) T cells specific for a viral antigen is perhaps 200 cells of the 10^8 CD8 T cells in a naïve mouse, far too low to provide immediate protection [7] (Table 1). To provide any protection, clonal expansion of these specific T cells is crucial. Over a seven day period of infection these 200 cells expand at an astonishing rate, in some cases reaching a ceiling of more than 10^7 cells in the spleen alone, representing a 4-5 log increase [7]. After clearing the infection, most of the expanded T cells die, but a memory population of up to 10^6 cells per spleen persist, representing a nearly 10^4-fold increase compared to a naïve animal [8]. Memory T cells localize in tissues where infections may occur and respond to antigen more rapidly and more robustly than naïve T cells. Hence, memory can be attributed to greater numbers of antigen-specific T cells as well as more potent responses by each T cell. Considering the tiny number of antigen-specific T cells in a naïve animal, clonal expansion clearly plays a particularly important role in providing immunity to reinfection.

It has been appreciated for some time that NK cells display some features of adaptive immune cells [4]. Different NK clones display different sets of stimulatory and inhibitory receptors, for example, creating a repertoire of specificities that is normally considered a feature of adaptive immunity, and NK cells show specific self-tolerance that must be

established in each individual. When exposed to an antigen recognized by only a subset of NK cells, that subset “clonally expands” over a several day period [9]. And the possibility that NK cells have a form of memory was in fact suggested by several early reports, including a very early study of bone marrow graft rejection [10], and later studies showing that NK cells in naïve animals exist in a relatively inactive state, and must be pre-activated to attain a sensitized state that provides more potent protection [11]. Sensitization could be induced by exposing NK cells in vivo to a sensitive tumor target cell [11] or to agonists that nonspecifically activate NK cells (such as the innate immune stimulus poly I:C) [12], or in vitro to cytokines such as IL-2 or IL-15 [13].

Though long recognized, these “adaptive” features of NK cells seemed much less pronounced in NK cells than in T or B cells, and their significance has not been strongly emphasized. The NK repertoire is dramatically more limited than that of T cells or B cells, for example, and specific clonal expansion seemed to be much less robust. The sensitized state of activated NK cells was not thought to last long enough to justify the term “memory”. Among the new reports on NK memory, Cooper et al. showed that NK cells preactivated with specific cytokines (IL-12 plus IL-18), and then transferred to recipient mice, persist for several weeks in a highly sensitized state relative to similarly transferred naïve NK cells [3]. The duration of the sensitized state was striking (though the duration shown was much less than that of memory T cells), and would be expected to provide enhanced protection for a sustained period after an initial insult that activates NK cells. This form of memory was not specific, however, and did not require NK cell proliferation.

Sun et al now document the potential for dramatic specific clonal expansion of NK cells in mice infected with mouse cytomegalovirus (MCMV), and for persistence of expanded NK cells as memory cells [2]. The activating NK receptor Ly49H, expressed by ~50% of NK cells, is specific for the viral m157 protein and is required for NK control of MCMV. Ly49H + NK cells specifically expand only 3-10 fold in infected mice [9], but Sun et al now show that up to 1000-fold expansions could be achieved if the number of starting Ly49H+ NK cells was limited substantially by transferring small numbers of NK cells to mice with defective NK cells before infection [2] (Table 1). After some decay in numbers, expanded numbers of NK cells persisted for weeks after viral clearance and exhibited a sensitized phenotype. Transfer experiments indicated that these “memory NK cells” were more protective against MCMV than were a comparable number of naïve NK cells. It is notable that the number of NK memory cells gradually decayed to approach the starting number, unlike memory CD8 T cells, which stabilize at a greatly expanded number compared to the frequency in naïve mice. Although not tested, it would be of interest to test the specificity of the memory. Considering that NK cells are generally equipped with several types of receptors that can be used with some independence, it is unlikely that the NK memory is highly specific.

It was impressive to observe such robust clonal expansion of Ly49H+ NK cells. It must be kept in mind, however, that the robust expansion was observed under artificial conditions where the starting numbers of specific NK cells were purposefully limited. The normal starting frequency of Ly49H+ NK cells (approximately 2×10^6 cells per naïve spleen) is so very high compared to typical frequencies of antigen specific T cells (~200 cells per naïve spleen) that clonal expansion is bound to be less important for mounting an effective response. Robust clonal expansion would presumably be of greater immunological benefit if rare NK cell specificities existed naturally in the preimmune NK cell population. Such rare specificities have not yet been documented.

Specific NK cell memory was previously proposed by O’Leary et al, and their results raise the possibility that rare NK cell specificities may exist [14]. That study examined contact

hypersensitivity responses, in which chemical agents that modify ("haptenate") proteins are painted on the skin, and cause immune swelling reactions that were previously thought to be mediated exclusively by T cells. O'Leary et al showed that NK cells can also mediate the response. Surprisingly, NK cell contact hypersensitivity is hapten-specific. Exposure of mice to one hapten sensitized them to respond strongly later when re-exposed to the same hapten, but not to a different hapten, and vice-versa. Memory lasted for weeks, and could be transferred to a naïve animal with small numbers (10^5) of purified NK cells from a sensitized donor. While other interpretations are possible, the data raise the specter that hapten-specific NK cells were initially relatively rare in the naïve NK cell pool, and expanded in the memory pool. However, receptors expressed only rarely by NK cells have not been defined to date. The contact hypersensitivity system, an in vivo reaction that involves poorly-defined haptenated cell surface "antigens", is not ideal for defining novel recognition receptors.

The new data mark an evolution from the view that NK cells respond de novo to each insult. The sustained sensitization of NK cells as a result of cytokines or infection at the least constitutes a form of hazy, fairly short-term memory, wherein a previous encounter ensures that NK cells will for a period of weeks or months lash out vigorously when exposed to the same or a different insult. The clonal expansion data and contact hypersensitivity data indicate that more specific NK memories exist as well, but a detailed understanding of how this works, and how important it is, awaits data that clarifies whether novel NK receptors, or specificities, exist.

References

1. Medzhitov R. Recognition of microorganisms and activation of the immune response. Nature 2007;449:819–826. [PubMed: 17943118]
2. Sun JC, Beilke JN, Lanier LL. Adaptive immune features of natural killer cells. Nature 2009;457:557–561. [PubMed: 19136945]
3. 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
4. Raulet DH. Interplay of natural killer cells and their receptors with the adaptive immune response. Nat Immunol 2004;5:996–1002. [PubMed: 15454923]
5. Dorshkind K, Pollack SB, Bosma MJ, Phillips RA. Natural killer (NK) cells are present in mice with severe combined immunodeficiency (scid). J. Immunol 1985;134:3798–3801. [PubMed: 3989296]
6. Lanier LL. Up on the tightrope: natural killer cell activation and inhibition. Nat Immunol 2008;9:495–502. [PubMed: 18425106]
7. Murali-Krishna K, Altman JD, Suresh M, Sourdive D, Zajac A, Miller J, Slansky J, Ahmed R. Counting antigen-specific CD8 T cells: a reevaluation of bystander activation during viral infection. Immunity 1998;8:177–187. [PubMed: 9491999]
8. Kaech SM, Wherry EJ, Ahmed R. Effector and memory T-cell differentiation: implications for vaccine development. Nat Rev Immunol 2002;2:251–262. [PubMed: 12001996]
9. Dokun AO, Kim S, Smith HR, Kang HS, Chu DT, Yokoyama WM. Specific and nonspecific NK cell activation during virus infection. Nat Immunol 2001;2:951–956. [PubMed: 11550009]
10. Cudkowicz G, Stimpfling JH. Induction of immunity and of unresponsiveness to parental marrow grafts in adult F1 hybrid mice. Nature 1964;204:450–453. [PubMed: 14232531]
11. Glas R, Franksson L, Une C, Eloranta ML, Ohlen C, Orn A, Karre K. Recruitment and activation of natural killer (NK) cells in vivo determined by the target cell phenotype: An adaptive component of NK cell-mediated responses. Journal of Experimental Medicine 2000;191:129–138. [PubMed: 10620611]
12. Gidlund M, Orn A, Wigzell H, Senik A, Gresser I. Enhanced NK cell activity in mice injected with interferon and interferon inducers. Nature 1978;273:759. [PubMed: 566386]

13. Carson WE, Giri JG, Lindemann MJ, Linett ML, Ahdieh M, Paxton R, Anderson D, Eisenmann J, Grabstein K, Caligiuri MA. Interleukin (IL) 15 is a novel cytokine that activates human natural killer cells via components of the IL-2 receptor. J Exp Med 1994;180:1395–1403. [PubMed: 7523571]

14. O'Leary JG, Goodarzi M, Drayton DL, von Andrian UH. T cell- and B cell-independent adaptive immunity mediated by natural killer cells. Nat Immunol 2006;7:507–516. [PubMed: 16617337]

Table 1

Comparison of NK cell and T cell response, and associated memory

Cell type	Precursor frequency (cells per spleen)	Antigen-induced expansion (normal conditions)	Persistence of memory	Specificity of memory
CD8 T cells	~200	10^4-10^5 fold	years	high
NK cells (eg. Ly49H+)	~2×10^6	3-10 fold (10^3 fold possible)	weeks-months	low?