Natural killer cells might adapt their inhibitory receptors for memory

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Traditional teaching in immunology tells us that antigen-specific recall responses are the realm of adaptive immunity. However, over the past decade, there has been increasing evidence and interest in the concept of innate immune memory. Natural killer (NK) cells are the founding member of the innate lymphocyte family, initially recognized more than 40 y ago for their ability to kill tumor targets without prior sensitization across MHC haplotypes. Since that time, germline-encoded NK cell receptors (NKRs) that recognize class I MHC (MHC-I) molecules and deliver "activating" or "inhibitory" signals dictating NK cell education and function have been found. A decade ago, NK cells were discovered to have the capacity for specific recall responses to diverse antigens, akin to those seen in T cells. The mechanism by which NK cells specifically recognize a wide range of antigens is unknown. In PNAS, Wight et al. (1) provide evidence that inhibitory NKRs might recognize antigen in the context of MHC-I molecules to mediate NK cell memory responses.

The first evidence for NK cell memory responses came when O'Leary et al. (2) demonstrated that a subset of liver NK cells mediates hapten-specific contact hypersensitivity (CHS) in Rag-deficient mice. Adoptive transfer experiments of purified NK cells demonstrated specificity of long-lived NK cell recall responses (2, 3). Three major types of NK cell memory or memory-like responses are now recognized: antigen-specific responses, CMV-adapted memory (in the mouse reliant on a virally encoded ligand interacting with a germline-encoded NK cell-activating receptor), and cytokine-induced antigen-independent memory-like responses (2, 4–7). Wight et al. (1) focus on antigen-specific NK cell memory and investigate whether Ly49 inhibitory NKRs can recognize antigen.

Antigen-specific NK cell memory has been demonstrated for a wide variety of antigens, including haptens, viral-like particles, and in the context of infections such as influenza, vaccinia, and others (reviewed in ref. 7). Memory NK cell responses can be protective in vaccination models, and Paust et al. (3) showed that administration of inactivated vesicular stomatitis virus induced an NK cell population that conferred specific protection to lethal viral challenge in T and B cell-deficient mice. Prior sensitization with monobenzone enhanced NK cell antitumor responses to B16 melanoma tumor cells, thought to be due to monobenzone "haptenizing" melanocyte antigens (8). Although the concept of antigen-specific responses, independent of Rag-mediated receptor recombination, challenges the paradigms of immunological memory, results have been recapitulated in multiple independent studies in the mouse, and there is evidence of their existence in nonhuman primates and humans (7, 9). The mechanisms by which NK cells, which are not known to somatically rearrange receptors, can sense a wide array of antigens are unknown.

Sensitization	Challenge	Response?
SIINFEKL	SIINFEKL	+ CHS
SIYRRLGL	SIYRRLGL	+ CHS
SIYNFEKL	SIYNFEKL	+ CHS
SIYRRLGL	SIINFEKL	No swelling
SIYNFEKL	SIINFEKL	No swelling
SIYNFEKL	SIYRRLGL	+CHS

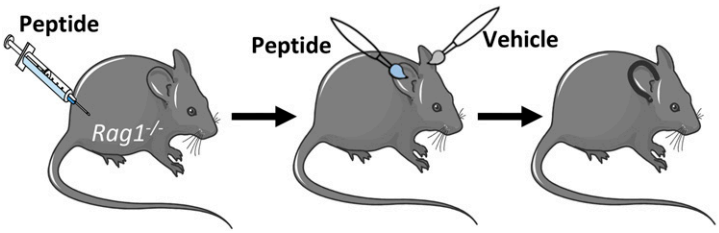

Fig. 1. Rag-deficient mice were sensitized with peptides by Wight et al. (1) to test NK cell antigen-specific recall responses in a model of CHS. While previous studies demonstrated strict specificity for antigens, these investigators found that peptides sharing the same amino acids at positions 2 and 3, thought to bind NK cell Ly49C, exhibited cross-reactivity.

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Wight et al. (1) used the CHS model to test whether inhibitory Ly49 NKRs are responsible for antigen recognition. In this model, haptens (small molecules that alter self-proteins) applied to the skin elicit specific recall responses in previously sensitized mice, measured as ear swelling and inflammatory infiltrate. While classically used to interrogate adaptive immunity, when done in Rag-deficient hosts lacking T and B cells, memory NK cell responses are detected (2). Wight et al. (1) used a genetic model in which Ly49 expression is severely reduced on a Rag1-deficient background to demonstrate that CHS to haptens and peptide antigens is not observed but can be rescued by transgenic expression of the inhibitory Ly49I receptor. Although suggestive of a requirement for Ly49 to recognize antigen, the model is complicated by the fact that in the absence of Ly49 receptors, NK cells are known to have poor function due to a lack of education (10). It was originally shown by O'Leary et al. (2) that NK cells with inhibitory Ly49 specific for self-MHC mediate CHS, which was thought to be related to an education requirement. Thus, it is difficult to distinguish the requirement for Ly49 for education versus memory response. Wight et al. (1) address this in two ways. First, they used $F(ab')_2$ fragments of antibody to Ly49 receptors to inhibit Ly49 interactions without depleting cells. They blocked either Ly49C/I inhibitory receptors, which recognize MHC-I in C57BL/6 mice, or Ly49G inhibitory receptors, which do not have an MHC-I ligand in C57BL/6 mice. Results show that blocking inhibitory Ly49C/I, but not Ly49G, during either sensitization or challenge abrogated hapten-mediated CHS in Rag1-deficient mice. Second, they used MHC-I–deficient animals to demonstrate a lack of NK cell-mediated CHS, although this is again challenging since NK cells in this model are functionally deficient at baseline due to an education defect. However, they then tested MHC-I– or MCH-II–restricted ovalbumin peptides in a CHS model, demonstrating that antigen presented by MHC-I (which binds Ly49s), but not by MHC-II, can elicit an NK-mediated CHS response. Together, these results suggest that antigen presentation by relevant MHC-I and inhibitory Ly49 is required for NK cell antigen recall responses.

The authors hypothesize that because Ly49C binding has been reported to be sensitive to residues 2 and 3 of MHC-I antigen, there would be cross-reactivity of CHS responses to antigens with the same amino acids in these residues (1). Having demonstrated that peptides presented by MHC-I, but divergent at residues 2 and 3, elicit specific NK-mediated CHS responses without cross-reactivity, they tested a hybrid peptide that shares residues 2 and 3. Here, Wight et al. (1) observed cross-reactivity (i.e., mice sensitized with SIYNFEKL had a CHS response when challenged with SIYRYYGL, but not with SIINFEKL) (Fig. 1). Notably, this experiment is the first report of antigenic cross-reactivity in NK cell memory. These findings suggest that it may be possible to induce NK memory responses to a wide range of antigens by taking advantage of shared residues, although this will require additional testing. Lastly, they vaccinated Rag1-deficient mice with ovalbumin, followed by administering a melanoma tumor expressing ovalbumin protein. This vaccination strategy provided significant protection from tumor, consistent with prior studies using vaccination strategies targeting NK cells (3, 7, 8).

Many questions remain about the mechanism by which Ly49 receptors on NK cells might specifically recognize antigen in the context of MHC-I. Wight et al. (1) propose several potential mechanisms, including alternative splice variants of Ly49 molecules and fine-tuning of the assembled receptor to discriminate antigen, citing a similar mechanism in a tunicate species. This remains untested, and it will be important to determine the exact mechanism of Ly49 recognition of antigens in future studies.

The study by Wight et al. (1) suggests that inhibitory NKRs positively regulate memory and NK cell function. It may seem contradictory that an inhibitory signal would result in a more activated state; however, such a concept is not new to NK cell biology. Kim et al. (10) showed that inhibitory NKRs regulate NK cell education by a process termed "licensing," in which only those NK cells bearing inhibitory NKRs capable of recognizing self-MHC acquire functional competence. Bern et al. (11) used a genetic model to demonstrate that immunoreceptor tyrosine-based inhibition motif (ITIM) signaling is required for licensing. One possible explanation for this is that inhibitory receptors directly confer licensing through the same signaling motif required for effector inhibition. That the same receptor could confer outcomes perceived as positive or negative is not unprecedented, as demonstrated by the T cell receptor, which confers both positive and negative selection. Thus, NK cell education by inhibitory NKRs points to their diverse function and suggests that ITIM signaling is not necessarily a priori inhibitory with respect to cellular function. The original report of antigen-specific memory demonstrated that only apparently licensed NK cells with inhibitory NKRs recognizing self-MHC could mediate antigen-specific memory (2). Whether this is due to a lack of education or the inability to specifically recognize in the absence of inhibitory Ly49, as suggested by the results from Wight et al. (1), is uncertain. As the current study did not investigate ITIM and NKR signaling, it is unknown whether there is a mechanistic link between the signals required for NK cell education and memory formation.

Wight et al. provide evidence that inhibitory NKRs might recognize antigen in the context of MHC-I molecules to mediate NK cell memory responses.

Another question is whether lymphocytes capable of recognizing a wide range of antigens are bona fide NK cells or represent an alternate innate lymphoid cell (ILC) population. NK cells are the original ILCs, a family of lymphocytes that includes multiple subsets of lymphocytes defined by their lack of dependence on Rag-mediated receptor recombination and categorized based on functional attributes, sites of tissue residence, and differentiation requirements (12). Phenotypically, antigen-specific memory NK cells appear to be bona fide NK cells based on cell-surface expression of NK1.1 or DX5 and Ly49 receptors; however, Wight et al. (1) show that NK-mediated CHS is perforin independent, suggesting that cytotoxic function is not required for memory in this context. Cytotoxic capacity is one of the distinguishing functional attributes of NK cells that differentiates them from ILCs. The transcription factors required for differentiation of antigen-specific NK cell memory have not been tested, and this information will provide insight into their place in ILC biology.

There has been interest in the NK cell field to harness NK cell memory for vaccine strategies. While the study by Wight et al. (1) suggests that targeting inhibitory NKRs may be an effective approach, one caveat is that there are significant differences in the structure of mouse and human NKRs. Interestingly, both mice and humans appear to have evolved with structurally divergent NKRs that serve to both recognize MHC and regulate NK cell function—an example of convergent evolution (13). Ly49 receptors in mice are lectinlike receptors that express intracellular ITIM domains (inhibitory) or partner with adapter proteins with activation motifs (activating).

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The major family of human NKRs, killer cell Ig-like receptors (KIRs), similarly either express cytoplasmic ITIM domains or partner with adapter proteins. Mouse and human inhibitory NKRs both down-regulate NK cell cytotoxicity when engaged with self-MHC and mediate NK cell education. Thus, while they serve similar roles in NK cell biology, it is unknown whether KIRs might recognize antigen.

The finding that inhibitory Ly49 receptors appear to recognize antigen in the context of MHC-I provides the first potential clue to the receptor basis of diverse antigen-specific NK cell recall responses. Many questions remain regarding the signaling required for memory NK cell differentiation and how receptors that are germline encoded acquire the ability to recognize specific antigen. Insights into these mechanisms have the potential to influence our understanding of NK cells in human disease and vaccination strategies targeting NK cells.

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1 Wight A, et al. (2018) Critical role for the Ly49 family of class I MHC receptors in adaptive natural killer cell responses. *Proc Natl Acad Sci USA* 115:11579–11584.
2 O'Leary JG, Goodarzi M, Drayton DL, von Andrian UH (2006) T cell- and B cell-independent adaptive immunity mediated by natural killer cells. *Nat Immunol* 7:507–516.
3 Paust S, et al. (2010) Critical role for the chemokine receptor CXCR6 in NK cell-mediated antigen-specific memory of haptens and viruses. *Nat Immunol* 11:1127–1135.
4 Cooper MA, et al. (2009) Cytokine-induced memory-like natural killer cells. *Proc Natl Acad Sci USA* 106:1915–1919.
5 Sun JC, Beilke JN, Lanier LL (2009) Adaptive immune features of natural killer cells. *Nature* 457:557–561.
6 Cooper MA, Yokoyama WM (2010) Memory-like responses of natural killer cells. *Immunol Rev* 235:297–305.
7 Paust S, Blish CA, Reeves RK (2017) Redefining memory: Building the case for adaptive NK cells. *J Virol* 91:e00169-17.
8 van den Boorn JG, et al. (2016) Inflammasome-dependent induction of adaptive NK cell memory. *Immunity* 44:1406–1421.
9 Reeves RK, et al. (2015) Antigen-specific NK cell memory in rhesus macaques. *Nat Immunol* 16:927–932.
10 Kim S, et al. (2005) Licensing of natural killer cells by host major histocompatibility complex class I molecules. *Nature* 436:709–713.
11 Bern MD, et al. (2017) Immunoreceptor tyrosine-based inhibitory motif-dependent functions of an MHC class I-specific NK cell receptor. *Proc Natl Acad Sci USA* 114:E8440–E8447.
12 Vivier E, et al. (2018) Innate lymphoid cells: 10 years on. *Cell* 174:1054–1066.
13 Yokoyama WM (2013) Natural killer cells. *Fundamental Immunology*, ed Paul W (Lippincott Williams & Wilkins Philadelphia), 7th Ed, pp 395–430.

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