Focus: Molecular Memory

Immunological memory within the innate immune system

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Abstract

Immune memory has traditionally been the domain of the adaptive immune system, present only in antigen-specific T and B cells. The purpose of this review is to summarize the evidence for immunological memory in lower organisms (which are not thought to possess adaptive immunity) and within specific cell subsets of the innate immune system. A special focus will be given to recent findings in both mouse and humans for specificity and memory in natural killer (NK) cells, which have resided under the umbrella of innate immunity for decades. The surprising longevity and enhanced responses of previously primed NK cells will be discussed in the context of several immunization settings.

Keywords immunity; innate immunity; memory; NK cells

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Introduction—a brief history of immunological memory

Immune memory is a function of specificity and longevity—the ability of antigen-specific cells of the immune system to recognize and “remember” pathogens previously encountered and to produce a qualitatively and quantitatively different response (i.e., faster or more robust) than the first encounter. From the discovery of vaccination against smallpox by Edward Jenner (in 1796) to the implementation of Louis Pasteur’s rabies vaccine (in 1885) and passive immunization developed by Shibasaburo Kitasato and Emil von Behring (in 1890), the ability to elicit immune memory responses to confer protection against pathogens had been attributed solely to antibodies and antibody-producing cells. It was not until the 1960s that Jacques Miller found a lymphocyte subset dependent on the thymus, which could also play a role in the recognition of antigens (Miller & Mitchell, 1967; Mitchell & Miller, 1968); since the 1980s, when the idea of T-cell memory first blossomed, we have a broad understanding of how memory CD8⁺ and CD4⁺ T cells are generated against infectious pathogens (Williams & Bevan, 2007; Swain *et al*,

2012; Mueller *et al*, 2013). Many of the current vaccination strategies against modern day scourges (such as HIV) are aimed at eliciting potent memory CD8⁺ T-cell responses when pathogens are encountered (Kaech *et al*, 2002; Ahmed & Akondy, 2011; Koup & Douek, 2011). Thus, until less than a decade ago, it was understood that only T and B cells of the adaptive immune system were capable of antigen specificity, clonal expansion, generation of memory cells, and the mounting of recall responses; however, the evidence presented in this review suggests that other cells of the immune system also possess these traits.

Evidence of immune memory in lower organisms

During the evolution of species, acquisition of adaptive immunity (and along with it immune memory) is thought to have occurred during the appearance of early jawed vertebrates, and involving mechanisms whereby diversity in the antigen receptor repertoire is attained. In the past decade, Max Cooper and his colleagues have identified and characterized primordial lymphocyte-like cell subsets and receptors within the surviving agnathans (or jawless fish, e.g., lamprey and hagfish) that resemble T and B cells and their receptors in higher vertebrates (Boehm *et al*, 2012). This “alternate” adaptive immune system consisting of variable lymphocyte receptors (VLRs) may provide a molecular mechanism to explain findings in the 1960s by Robert Good and his colleagues that sea lampreys can produce antigen-specific agglutinating antibody during immunization with the bacteria *Brucella abortus*, rapidly reject a second set of skin allografts, and develop delayed-type hypersensitivity (DTH) responses to Freund’s complete adjuvant containing *Mycobacterium tuberculosis* (i.e., tuberculin test) (Finstad & Good, 1964). Similar secondary immune responses were observed in hagfish and other primitive fishes ranging from elasmobranchs to holosteans to teleosts (Papermaster *et al*, 1964). Although the precise molecular processes behind the generation of VLR diversity remain to be determined, these discoveries suggest that mechanisms of immune memory may have existed in jawless fish at the time of vertebrate radiation (530 million years ago) preceding the Devonian period (when jawed vertebrates became common) (Fig 1).

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	Antigen Receptors	Mechanism of diversity	Antigen specificity	Immune memory
Higher vertebrates Jawed fishes, mouse, human	TCR BCR	V(D)J Recombination	Yes	Yes
Lower vertebrates Jawless fishes	VLRA VLRB VLRC	Leucine-rich repeats	Yes	Yes
Invertebrates Insects, worms, shellfish	Toll, DSCAM, others?	?	Yes?	Yes?

Figure 1. Mechanisms for antigen specificity and memory in vertebrates and invertebrates.

In higher vertebrates (from jawed fish to mammals), antigen specificity and immune memory are mediated by lymphocytes possessing rearranged antigen receptors. In lower vertebrates such as lampreys and hagfish (jawless fish), VLRs containing leucine-rich repeats could be responsible for a primitive form of adaptive immunity. In invertebrates (from shellfish to worms and insects), the diversity of antigen receptors is likely limited (for sensing of conserved pathogen-associated molecular patterns), but evidence exists for both specificity and memory. TCR, T-cell receptor; BCR, B-cell receptor; VLR, variable lymphocyte receptors; V(D)J recombination: randomly combines variable, diverse, and joining gene segments in vertebrate lymphocytes.

Of the animal kingdom, > 97% of known species are invertebrates, of which no form of adaptive immune system has thus far been identified. Because of the lack of evidence, many publications focused on invertebrate immunity will express how invertebrates are ideal for studying innate immunity in the absence of adaptive immunity. However, is there evidence for invertebrate immune “memory” of pathogens found in insects, worms, crustaceans, and sea sponges? Although this question was raised by several groups in the late 1990s, a study published in 2003 by Joachim Kurtz and colleagues directly investigated whether invertebrate memory exists in the copepod *Macrocyclus albidus* (a minute crustacean) against its natural pathogen, the parasitic tapeworm *Schistocephalus solidus* (Kurtz & Franz, 2003). Following a primary exposure to the parasite, the copepod became resistant to subsequent challenge with an antigenically similar tapeworm, providing one of the first lines of evidence for innate immune memory in a crustacean species (Kurtz & Franz, 2003; Kurtz, 2005). The following year, Eric Loker and colleagues demonstrated that the freshwater snail *Biomphalaria glabrata* contained extraordinary somatic recombinatorial diversity at the immunoglobulin superfamily domains of hemolymph proteins (Zhang et al, 2004), suggesting primitive but parallel processes in invertebrates for generating diversity and specificity analogous to

antigen receptor gene rearrangement and somatic hypermutation in mammals. Related to these findings, George Dimopoulos and colleagues recently described a molecular mechanism whereby alternative transcript splicing produces pathogen-specific splice variant repertoires of the hypervariable immune effector and pattern recognition receptor Dscam (Down syndrome cell adhesion molecule), which protects *Anopheles gambiae* mosquitoes against malaria (*Plasmodium falciparum*), allowing it serve as a major vector for transmission of the parasite between humans (Dong et al, 2012).

Although the fruit fly is thought to solely rely on innate defense mechanisms through self-/non-self-discrimination involving the Toll pathway (Hoffmann & Reichhart, 2002; Janeway & Medzhitov, 2002; Lemaitre & Hoffmann, 2007), it was not known whether activation of this pathway in phagocytes could result in resistance against subsequent pathogen exposure. In a study published in 2007, David Schneider and colleagues demonstrated that *Drosophila melanogaster* which were first injected with a sublethal dose of *Streptococcus pneumoniae* or *Beauveria bassiana* (a natural fruit fly pathogen) did not succumb to a subsequent bacterial challenge, and established that the Toll pathway was essential for mediating secondary responses and protection (Pham et al, 2007). In 2009, two separate studies by Kurtz and colleagues demonstrated specific priming of resistance against bacteria *Bacillus thuringiensis* and *Escherichia coli* in the red flour beetle *Tribolium castaneum* and the woodlouse *Porcellio scaber* (Roth & Kurtz, 2009; Roth et al, 2009). They found that immune specificity and memory existed in red flour beetles and woodlouse previously primed with heat-killed bacteria, which could survive a subsequent exposure to the same bacteria that was used in priming, but not against an unrelated bacteria, by increasing hemocyte phagocytosis during secondary challenge. Similarly, Carolina Barillas-Mury and colleagues pre-exposed *A. gambiae* mosquitoes to *P. falciparum* and observed enhanced immunity upon parasite reinfection, with the protection attributed to increased circulating granulocyte numbers following primary infection (Rodrigues et al, 2010). Numerous other studies throughout the years using insect models have demonstrated the ability of invertebrates to mediate specific immunity against secondary bacterial and fungal exposure (Faulhaber & Karp, 1992; Moret & Siva-Jothy, 2003; Bergin et al, 2006; Sadd & Schmid-Hempel, 2006). Altogether, the studies above suggest that innate immune cells in many simpler organisms can be primed by previous infections and mount stronger recall responses upon homologous pathogen challenge (Fig 1).

Interestingly, recent studies using worm models suggest that the memory in the nervous system can directly influence innate immune responses (Mahajan-Miklos et al, 1999; Remy & Hobert, 2005; Zhang et al, 2005; Anyanful et al, 2009; Shirayama et al, 2012; Inoue et al, 2013). The nematode *Caenorhabditis elegans* was shown to modulate its olfactory preferences after initial exposure to the pathogenic bacteria *Pseudomonas aeruginosa* and *Escherichia coli* such that it will avoid subsequent exposure to toxic bacteria for host preservation (Mahajan-Miklos et al, 1999; Pujol et al, 2001; Zhang et al, 2005; Anyanful et al, 2009), suggesting that adaptation by associative learning (i.e., “olfactory imprinting”) may have been evoked via immune networks activated during first encounter with pathogen. In animal behavior studies, this form of learning or conditioning to discriminate between external stimuli is known as an avoidance response. The cellular and molecular mechanisms responsible for conditioning worms toward avoidance behavior may

operate at multiple levels: in the dopaminergic neurons and innate immune cells themselves (Mahajan-Miklos *et al*, 1999; Anyanful *et al*, 2009), the effector molecules and receptors that transmit or sense signals between these cells (Remy & Hobert, 2005; Zhang *et al*, 2005; Inoue *et al*, 2013), or even through “epigenetic memory” mediated by piRNAs that can discriminate non-self- from self-RNA (Shirayama *et al*, 2012). Although much remains to be understood regarding nervous system–immune system cross-talk, studies such as these suggest that efferent neural circuits that sense injury and infection may also be able to modulate immune responses and influence immune memory (Tracey, 2009).

Early evidence of memory in the innate immune system

Our understanding of the innate immune system has evolved from Elie Metchnikoff's early descriptions of phagocytic cells (which he called macrophages) and granulocytes (such as neutrophils, eosinophils, basophils) in starfish (1880s) to Ralph Steinman and Zanvil Cohn's discovery of dendritic cells in mice nearly a century later (Steinman & Cohn, 1973), to the complex cross-talk that occurs between innate immune cells and those of the adaptive immune system which we are still trying to delineate today. At around the same time that dendritic cells were described, a killer lymphocyte subset lacking T- and B-cell receptors (thus referred to as “null” cells among other names) was shown to mediate rapid cytotoxicity against tumors and virally infected cells in the absence of prior sensitization, prompting Eva Klein and others to coin the phrase “natural killer” (NK) in 1975 (Herberman *et al*, 1975a,b; Kiessling *et al*, 1975a,b). Since that time, the placement of NK cells within the immune system has been somewhat of a conundrum. Are NK cells adaptive because they resemble lymphocytes and function like CD8⁺ T cells, or are they innate because they respond rapidly and lack rearranged antigen (RAG)-generated receptors? Although the work of Irving Weissman and colleagues in 1997 did not resolve the confusion, they formally demonstrated that NK cells comprised a third lineage of lymphocytes, being derived from the same lymphocyte progenitor as T and B cells (Kondo *et al*, 1997); yet, to this day, NK cells are placed in immunology textbooks under the heading of innate immunity because of their rapid responsiveness and lack of RAG-generated receptors (Paul, 2008; Abbas *et al*, 2012, 2014; Murphy *et al*, 2012; Owen *et al*, 2013). In recent years, additional populations of “innate” lymphocytes have been identified, with some that require expression of the RAG recombinases for their development (NKT cells, $\gamma\delta$ T cells, CD8 $\alpha\alpha$ T cells, mucosa-associated invariant T cells or MAIT, and other “non-classical” T cells; along with B1 B cells and marginal zone B cells) and others that do not require RAG such as the newly identified innate lymphoid cells (ILC1, ILC2, and ILC3) (Spits *et al*, 2013), further blurring the formerly concrete parameters that separated innate from adaptive immunity. Although the textbook definition of innate immunity contains boundaries that may now be in question, cells of the innate immune system have been generally thought to respond rapidly and non-specifically and be short-lived. Thus, adaptive immune features such as antigen specificity, clonal expansion, and memory were not thought to exist in innate lymphocytes like NK cells; however, in the past decade, a plethora of studies have emerged that challenge this dogma.

Although NK cells and their functions were not uncovered until the early 1970s and NK-cell recognition of “missing self” described by Klas Karre until 1986 (Karre *et al*, 1986; Ljunggren & Karre, 1990), evidence for NK-cell memory has existed since as early as the 1960s—before NK cells were first described. In a 1964 study performed by Gustavo Cudkowicz and Jack Stimpfling that was one of the first to describe the phenomenon known as F1 hybrid resistance (shown in later years to be NK cell mediated), F1 hybrid mice that had been pre-treated with a parental bone marrow graft were able to more rapidly reject a second graft from the same parent compared to a graft from the other parent (Cudkowicz & Stimpfling, 1964) (Fig 2), suggesting that NK cells can retain a memory of past antigen encounter and mediate more robust secondary responses. In more recent years, NK cells have been shown to demonstrate both specificity and memory against a wide range of antigens (pathogens and chemical haptens) and stimuli (pro-inflammatory cytokines and lymphopenia) (O'Leary *et al*, 2006; Cooper & Yokoyama, 2010; Paust & von Andrian, 2011; Sun & Lanier, 2011; Vivier *et al*, 2011). Adaptive immune features of NK cells will be discussed in detail in the sections to follow.

Evidence of NK-cell memory against pathogens

If immunological memory evolved as a mechanism of host defense within the NK-cell compartment, one might hypothesize that memory against pathogens which have co-evolved alongside mammalian species would make the most sense, given the limited repertoire of antigen receptors present on the surface of NK cells (relative to T and B cells) (Lanier, 2005). Cytomegalovirus (CMV), a large double-stranded DNA virus from the β -herpesvirus family, constitutes such a pathogen, where NK-cell receptors in human and mice have evolved to specifically recognize HCMV- and MCMV-encoded proteins, respectively (Vidal & Lanier, 2006; Sun & Lanier, 2009). Indeed, one of the first reports of NK-cell memory against a pathogen was against MCMV (Fig 3). Lewis Lanier and colleagues demonstrated that NK cells expressing the activating Ly49H receptor, which recognizes the MCMV-encoded m157 antigen and signals through the DAP12 adaptor molecules, undergo a clonal-like expansion during MCMV infection and generate long-lived memory NK cells (Sun *et al*, 2009). These memory Ly49H⁺ NK cells were capable of self-renewal, preferentially resided in spleen and non-lymphoid sites for several months, and possessed the ability to mount prolific secondary and tertiary responses (Sun *et al*, 2009, 2010). The programming of NK-cell memory was demonstrated to involve early pro-inflammatory cytokine signals operating through IL-12 and STAT4 (Sun *et al*, 2012) along with suppression of Noxa and SOCS1 by microRNA-155 (Zawislak *et al*, 2013), whereas the maintenance of memory NK cells required continued IL-15 signals (Firth *et al*, 2013). In addition, the activating receptor DNAM-1 (CD226) cooperates with Ly49H to augment the expansion and memory phase of the NK-cell response by signaling through Fyn and PKC η (Nabekura *et al*, 2014). Similarly in human studies, several groups published longitudinal clinical studies demonstrating that NK cells expressing the activating CD94/NKG2C receptor robustly expand following HCMV infection or reactivation and can persist for years (Guma *et al*, 2006a,b; Kuijpers *et al*, 2008; Lopez-Verges *et al*, 2010, 2011; Della Chiesa *et al*, 2012; Foley *et al*, 2012a,b). Thus,

Figure 2. First F1 hybrid resistance studies also suggest natural killer (NK)-cell memory and recall responses.

In the 1960s, studies demonstrated that parental (A or B) bone marrow or skin grafts given to F1 progeny (A × B) mice were rejected; this observation was called F1 hybrid resistance. In these studies, rejection of a second bone marrow graft occurred more rapidly if the graft was derived from the same parent as the previous graft, suggesting that "pre-treatment" or priming of NK cells results in more potent recall responses during "challenge". (A) Schematic of experimental design by Gustavo Cudkowicz and Jack Stimpfling published in *Nature* in 1964. (B) Chart shows results from their experiments. A, B10 mouse strain; B, B10.D2 mouse strain.

CMV-specific NK cells in mouse and human possess characteristics previously attributable to CD8⁺ T cells of the adaptive immune system (Sun & Lanier, 2011), suggesting the possibility of a convergent evolution in these anti-viral immune responses. Future studies will determine the precise molecular signals that promote memory NK-cell generation during CMV infection in mouse and human.

The challenges with drawing conclusions from the longitudinal HCMV studies involve the nature of the infection or reactivation of virus in cancer patients, where patients are already heavily immunosuppressed and detection of HCMV infection immediately results in treatment with anti-viral drugs. Perhaps the first report in humans of NK-cell clonal expansion and memory following acute infection (without pharmaceutical intervention) may be a retrospective study performed by Hans-Gustaf Ljunggren and colleagues. In this study where the virus runs its natural course in humans, prolific expansion and sustained levels of elevated NK-cell numbers were detected in infected individuals during a 2007 outbreak of hantavirus in northern Sweden (Bjorkstrom *et al*, 2011). A similar proliferative burst of NK cells followed by persisting cell numbers or enhanced function have been observed in the setting of HBV, HCV, and chikungunya virus infection in humans (Petitdemange *et al*, 2011; Beziat *et al*, 2012). Interestingly, it was the NKG2C⁺ NK-cell population that underwent clonal expansion during all of the viral infections, and this occurred only in individuals previously infected with HCMV.

NK-cell clonal proliferation and memory have also been reported in a wide range of viral models in mice, including vaccinia virus (Gillard *et al*, 2011), influenza virus (van Helden *et al*, 2012), and herpes simplex virus infection (Abdul-Careem *et al*, 2012). In addition, priming of RAG-deficient mice with specific antigens from influenza, vesicular stomatitis virus, and even human immunodeficiency virus (which does not naturally infect mice) resulted in protection against challenge with sensitizing virus (Paust *et al*, 2010), shown to be mediated by CXCR6-expressing NK cells residing in the liver. Finally, macaques previously infected with recombinant HIV

possess elevated NK-cell responses 5 years after immunization (K. Reeves, personal communication), suggesting that this feature of longevity and memory in NK cells is conserved across mammalian species. Further studies are required to determine whether antigen specificity and immune memory in NK cells are imprinted at a genetic level by initial stimulation, and how potential epigenetic modifications are retained and inherited by progeny after multiple cell divisions.

Evidence of NK-cell memory in non-pathogenic contexts

Although evidence for NK-cell memory has been provided in the setting of various infections in mouse and humans, other mechanisms have been reported by which NK cells become capable of mounting recall responses. Ulrich von Andrian and colleagues first demonstrated that NK cells possess antigen specificity and DTH responses against chemical haptens such as DNFB and oxazolone (O'Leary *et al*, 2006). RAG-deficient mice (which lack T and B cells) immunized with a hapten were later challenged with the same hapten and a DTH response was observed, but not when mice were challenged with a different hapten (Fig 4). The antigen-specific recall responses were not observed in mice where NK cells were ablated or in *Rag2*^{-/-} × *Il2rg*^{-/-} mice (lacking T, B, and NK cells), strongly suggesting that the DTH responses were NK cell mediated. In this model, the hapten-specific memory NK cells were found to reside in the liver, as the ability to mediate DTH responses was transferrable by adoptively transferring liver NK cells, but not spleen NK cells, from the sensitized mice to naïve mice.

Another setting where NK-cell memory and robust recall responses were observed was during exposure of NK cells to pro-inflammatory cytokines (in the absence of antigen receptor triggering) (Fig 4). Wayne Yokoyama and colleagues adoptively transferred IL-12- and IL-18-stimulated NK cells into naïve mice and tested their ability to respond to these same cytokines several weeks

Figure 3. Adaptive immune features of natural killer (NK) cells during viral infection.

During MCMV infection, the viral ligand m157 is expressed on the surface of infected cells and recognized by Ly49H-expressing NK cells. NK cells receiving Ly49H-mediated signals via DAP12, and IL-12 signals through STAT4, will undergo clonal expansion to generate a large population of effector Ly49H⁺ NK cells. Following a contraction phase that is governed by microRNA-155 and IL-15 signals, self-renewing memory NK cells are long-lived and can mount recall responses against subsequent viral infection.

later (Cooper *et al*, 2009). The cytokine-induced memory NK cells from both mouse and human were able to produce IFN- γ more robustly than resting NK cells when non-specifically activated by pro-inflammatory cytokines (Cooper *et al*, 2009; Romee *et al*, 2012). Thus, a rationale may exist for the pre-activation of NK cells using pro-inflammatory cytokines prior to use as an adoptive immunotherapy strategy for the treatment of infection and cancer. Indeed, Heidi Cerwenka and colleagues demonstrated the efficacy and sustained effector function of IL-12-, IL-15-, and IL-18-treated NK cells against previously established tumors (Ni *et al*, 2012). It remains to be determined whether tumor-specific NK-cell responses can be primed resulting in long-lived killer cells poised to better recognize and remove transformed cells.

Finally, NK cells will undergo rapid, but non-specific homeostatic proliferation during times of lymphopenia, thus when lymphocyte numbers are very low in the organism (Prlc *et al*, 2003; Ranson *et al*, 2003; Jamieson *et al*, 2004). This is thought to be mediated by cytokines from the common gamma receptor family (e.g., IL-2, IL-7, IL-15) in addition to the “empty space” that is present in lymphopenic animals because of the absence of lymphocytes. Lanier and colleagues demonstrated that NK cells placed into lymphopenic environments (*Rag2*^{-/-} *x* *Il2rg*^{-/-} mice or sublethally irradiated mice) could become long-lived cells (Sun *et al*, 2011). As long as 6 months after transfer of NK cells into lymphopenic mice, the long-lived cells were able to robustly respond to viral infection (Fig 4). A subsequent study demonstrated that treatment of NK cells with pro-inflammatory cytokines prior to adoptive transfer did not prove detrimental to their longevity (Keppel *et al*, 2013). Thus, although questions remain about whether antigen specificity is required for NK-cell memory in pro-inflammatory or lymphopenic settings, several pathogen-independent mechanisms have been documented by which NK cells can acquire the adaptive immune traits of longevity, self-renewal, and robust recall responses.

Outstanding questions in innate immune memory

Do additional innate subsets possess features of adaptive immunity? Similar to NK cells, $\gamma\delta$ T cells are thought to be “innate” in nature even though this subset of T cells require the RAG recombinases and possess restricted TCR repertoires. In a recent study, the late Leo Lefrancois and his colleagues demonstrated that a distinct subset of $\gamma\delta$ T cells preferentially residing in the intestinal mucosa will mount an immune response to oral *Listeria monocytogenes* infection and develop a multifunctional memory response against homologous bacteria challenge, but not against a heterologous bacterium (Sheridan *et al*, 2013). These pathogen-specific memory $\gamma\delta$ T cells were able to confer greater protection during *Listeria* challenge, demonstrating one of the key hallmarks of adaptive immunity. Future studies will determine whether other “innate lymphocytes” such as NKT cells, non-classical MHC class I (Qa-1, H2-M3, TL)-restricted T cells, MAIT, B1 B cells, and marginal zone B cells possess the ability to be long-lived and mount efficacious recall responses.

Do innate immune cells that do not require RAG or gene rearrangement for their development possess characteristics of adaptive immunity similar to NK cells? Although previous studies have demonstrated the lifespan of dendritic cells in lymphoid organs such as spleen and lymph nodes to be relative short (varying from 1 to 9 days depending on organ and activation status) (Kamath *et al*, 2000, 2002; Henri *et al*, 2001), derivatives of these cells such as Langerhans cells, which reside in the skin, are thought to possess a much longer half-life (at least 3 weeks) (Kamath *et al*, 2002; Shortman & Naik, 2007). Whether this longevity is modulated when pathogens or pathogen products are encountered through Toll-like or Nod-like receptors or intracellular sensors is not well characterized. Furthermore, if certain macrophages or dendritic cells (or their subsets) can become long-lived, can they be recalled in an antigen-specific

Figure 4. Priming of adaptive immune characteristics in natural killer (NK) cells by non-pathogenic stimuli.

Experimental schematic and observations of immune memory in the NK-cell compartment during priming with chemical haptens (A), exposure to pro-inflammatory cytokines (B), and in settings of lymphopenia (C). (A) NK-cell presence allows for a delayed-type hypersensitivity (DTH) response in mice when repeatedly treated with the same hapten. In mammals, DTH is defined as an hyperinflammatory recall response of the host to an antigen (challenge) that had been exposed earlier to the same antigen (immunization). For years, DTH to haptens (contact hypersensitivity, CHS) has been known as the archetype of adaptive T-cell-mediated immune responses (Vocanson *et al*, 2009). (B) NK cells memorize cytokine priming event, leading to increased IFN- γ production upon re-exposure to the same inflammatory cytokines as compared to unprimed cells. (C) NK cells are long-lived in lymphopenic mice and expand robustly upon MCMV infection.

manner when the same pathogen or pathogen product is re-encountered? Certainly from contact hypersensitivity studies in Rag2^{-/-} x Il2rg^{-/-} mice, no DTH responses were observed in mice lacking all lymphocytes (O'Leary *et al*, 2006; Paust *et al*, 2010), suggesting that haptens were unable to generate anamnestic responses within macrophage or dendritic cell subsets. However, several recent studies provide evidence that monocytes exposed to fungal and bacterial pathogens mount protective recall responses against reinfection (Kleinnijenhuis *et al*, 2012; Quintin *et al*, 2012), suggesting that even cells derived from the myeloid lineage in mammals may possess features of adaptive immunity (Bowdish *et al*, 2007; Benn *et al*, 2013; Monticelli & Natoli, 2013).

Lastly, does the newly characterized family of ILCs (which do not require RAG but originate from a Id2-dependent lymphoid progenitor common to NK-cell precursors) (Spits *et al*, 2013) possess the ability to become long-lived and mount secondary responses at mucosal sites such as the gut or skin? Future studies will undoubtedly reveal how many more of these additional

"innate" immune cell lineages contribute in an "adaptive" fashion during pathogen re-encounters. Beyond the immune system, does memory exist in other organs systems or cells of non-hematopoietic origin? And how do cells of these other organs systems (such as neurons) cross-talk with memory cells of the immune system to engage in host defense against pathogen or transformation? For several years now, the conceptual boundaries we have placed on innate and adaptive immunity have been blurred, causing us to re-evaluate the classical definitions of response kinetics, antigen specificity, longevity, and recall responses that we have used to compartmentalize the immune system. As we make significant progress in our understanding of innate immune memory, we must now begin to turn our attention toward the implications of such findings, and the application of this knowledge in the novel design of vaccines against a variety of pathogens and cancer.

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Conflict of interest

E.V. is a co-founder and shareholder in InnatePharma. The other authors have no conflicting financial interest.

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