nature immunology

Natural killer cell memory

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Natural killer (NK) cells are bone marrow–derived granular lymphocytes that have a key role in immune defense against viral and bacterial infections and malignancies. NK cells are traditionally defined as cells of the innate immune response because they lack RAG recombinase–dependent clonal antigen receptors. However, evidence suggests that specific subsets of mouse NK cells can nevertheless develop long-lived and highly specific memory to a variety of antigens. Here we review published evidence of NK cell–mediated, RAG-independent adaptive immunity. We also compare and contrast candidate mechanisms for mammalian NK cell memory and antigen recognition with other examples of RAG-independent pathways that generate antigen receptor diversity in non-mammalian species and discuss NK cell memory in the context of lymphocyte evolution.

Adaptive immunity is considered an exclusive feature of T cells and B cells, which use RAG recombinase–mediated recombination of variable, diversity and joining gene segments mediated by the RAG recombinase to generate a multitude of T cell antigen receptors and B cell antigen receptors[1]. Activation of the receptor by cognate antigen triggers the clonal selection and differentiation of short-lived effector cells and long-lived memory cells that, after antigen rechallenge, mount enhanced recall responses. Classic manifestations of antigen-specific memory, the hallmark of adaptive immunity, include hapten-induced contact hypersensitivity (CHS)[2,3] and other forms of delayed-type hypersensitivity (DTH)[4]. Immunological memory protects against recurrent infections and is the central goal of active vaccination[5]. In contrast to T cells and B cells, cells of the innate immune response, including natural killer (NK) cells, do not express RAG proteins and are therefore incapable of rearrangement of variable-(diversity)-joining gene segments. They detect infection by using a finite number of germline-encoded pattern-recognition receptors, which allow activated NK cells to directly destroy pathogen-infected cells and to secrete cytokines and other proinflammatory mediators, which promote immune responses by other cells of the immune response. However, these 'hardwired' responses were not expected to change between successive encounters with the same pathogen, as the ability to 'learn' and 'remember' is considered unique to cells of the adaptive immune response.

NK cells are bone marrow–derived small granular lymphocytes that can lyse target cells without prior sensitization[6,7]. Their functions are controlled by germline-encoded receptors that integrate activating and dampening signals[8], stimulation by cytokines and chemokines, and communication with other leukocytes, particularly dendritic cells (DCs)[9]. NK cells confer resistance to tumors and infection through several mechanisms, including cytokine production and cytotoxicity[8]. Their importance in human host immunity is highlighted in patients with isolated genetic deficiencies in NK cells who contract severe infections despite the presence of functional T cells and B cells[10].

Traditionally, NK cells are considered cells of the innate immune response; however, there is accumulating evidence that at least some subsets respond to certain antigens in a manner that has the hallmarks of adaptive immunity. The first evidence of this came from observations that mice deficient in T cells and B cells acquire antigen-specific immunological memory to hapten-based contact sensitizers that is mediated by a subset of primed, hepatic NK cells[11,12]. Subsequent work has demonstrated that NK cells also acquire long-lived memory of diverse viral antigens[13,14]. However, the mechanisms by which NK cells recognize haptens or viruses and how they develop and maintain selective memory to these challenges are largely unclear. Here we examine the evidence for NK cell–mediated, RAG-independent adaptive immunity and summarize emerging ideas about the generation, maintenance and function of memory NK cells.

Evidence of NK cell–mediated acquired immunity

So far, the features of adaptive immunity in NK cells have been investigated mainly with mouse models through the use of two different modes of challenge: hapten-induced CHS, and viral infection. It is unknown whether NK cells acquire memory of other challenges or whether NK cell memory arises in other species. Nevertheless, as we will discuss below, there is considerable evidence that mouse NK cells develop and retain specific memory of highly diverse antigens.

Haptens covalently modify self proteins, generating neoantigens that are recognized by clonally selected lymphocytes[15]. Hapten-induced CHS was thought to depend on T cells[16], although at least one published study has suggested that this may not always be the case[17]. Evidence of the involvement of NK cells in CHS has been provided by studies of mice deficient in recombination-activating genes 1 and 2 and mice of the severe combined immunodeficiency strain, which are deficient in T cells and B cells. These mice acquire sensitization-dependent antigen-specific memory to three molecularly distinct contact sensitizers: 2,4-dinitro-1-fluorobenzene (DNFB), 4-ethoxy-methylene-2-phenyl-3-oxazalin-5-one (oxazolone) and picryl chloride[11]. Each of these haptens elicits a vigorous CHS response that features the three hallmarks of adaptive immunity: the response is 'learned' (that is, it

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Published online 18 May 2011; doi:10.1038/ni.2032

requires at least one episode of sensitization); sensitizing antigens are 'remembered', as CHS responses can be elicited as late as 4 months after sensitization; and the ensuing memory is antigen specific (that is, CHS is elicited only when identical haptens are used for sensitization and challenge). Antigen-specific memory is observable in T cell– and B cell–deficient mice with different genetic backgrounds regardless of major histocompatibility complex (MHC) haplotype. NK cells are required and sufficient to elicit CHS responses[11], as antibody-mediated depletion of NK cells abolishes recall responses in mice deficient in recombination-activating gene 2 (*Rag2*$^{-/-}$ mice) and CHS responses are absent from *Rag2*$^{-/-}$ mice deficient in the interleukin 2 receptor γ-chain, which lack all lymphocytes, including NK cells[18,19], as well as in mice of the severe combined immunodeficiency–beige strain, in which NK cells are dysfunctional[20]. Notably, hapten-specific memory is readily conferred to naive mice by adoptive transfer of NK cells from sensitized donors[11,12], but only when the transferred NK cells are isolated from the liver of donors sensitized with the same hapten used for challenge[11,14]. In contrast, splenic NK cells from the same donors do not transfer antigen-specific memory to naive hosts, which indicates that hepatic NK cells are functionally distinct from NK cells in other tissues, a finding that is consistent with other studies of rodents[21,22] and humans[23].

Together, the observations noted above suggest that mice have NK cells in their liver that mediate long-lived, antigen-specific adaptive immunity independently of B cells and T cells. Hapten-specific memory is concentrated in a small subset (<10%) of hepatic NK cells that express CD11b, CD90 (Thy-1.2) and CD186 (CXCR6) but not CD3 or CD27 (refs. 11,12,14). In C57BL/6 and C57BL/10 mice, NK cells that express the lectin-type receptors Ly49C and/or Ly49I are more potent at transferring CHS responsiveness than are Ly49C^{-} or Ly49I^{-} NK cells. Ly49C and Ly49I are cognate inhibitory receptors for self MHC class Ia molecules in C57BL/6 and C57BL/10 mice. Recognition of self MHC class I during NK cell development is necessary for the 'licensing' of NK cells to exert full-fledged effector functions in response to certain challenges[24], so licensing may also be needed to mount optimal recall responses to haptens. However, every marker that correlates with recall activity in hepatic NK cells is also found on splenic NK cells, which cannot develop memory; this indicates that these molecules, alone or in combination, are insufficient to acquire or exert antigen-specific memory.

After being primed with a hapten, adoptively transferred memory NK cells persist in naive hosts and retain antigen specificity for at least 4 months. Moreover, memory NK cells can also be isolated from the livers but not the spleens of sensitized wild-type donors, and they survive and function in both *Rag2*$^{-/-}$ mice deficient in the interleukin 2 receptor γ-chain and wild-type recipients, which indicates that the presence of T cells and B cells compromises neither their generation nor their persistence or anatomical distribution. After challenge of sensitized mice, or in recipients of sensitized NK cells, memory NK cells are recruited to and/or retained at sites of antigen challenge in an antigen-specific manner[11,14].

Antiviral responses have also provided evidence of NK cell memory–like phenomena. NK cells can be sensitized to develop long-lived protective memory of mouse cytomegalovirus (MCMV)[13]. MCMV is useful for the study of NK cell–mediated immunity because NK cells in C57BL/6 mice express the activating receptor Ly49H, which recognizes the MCMV-encoded protein m157 (refs. 25,26). In MCMV-infected C57BL/6 mice, Ly49H^{+} NK cells undergo rapid population expansion in the spleen and liver[13]. This proliferative response is particularly pronounced when the number of Ly49H^{+} 'naive' precursors is experimentally diminished. After a subsequent contraction phase, a population of self-renewing MCMV-specific memory NK cells persists for several months. The MCMV-experienced NK cells have higher expression of KLRG1, CD43, Ly6C and Ly49H than do naive NK cells, and they degranulate and produce interferon-γ (IFN-γ) more efficiently after reactivation. When transferred into newborn mice, which are susceptible to MCMV, Ly49H^{+} memory NK cells confer approximately tenfold better protection than do naive Ly49H^{+} NK cells[13]. Unlike hapten-specific memory NK cells, the MCMV-experienced NK cells are not confined to the liver but reside in peripheral and lymphoid tissues throughout the body.

Subsequent studies have demonstrated that mouse NK cells can acquire long-lived memory of diverse viral antigens, including vesicular stomatitis virus (VSV), influenza A and human immunodeficiency virus type 1 (HIV-1)[14]. After sensitization of RAG-deficient C57BL/6 or BALB/c mice with ultraviolet light–inactivated VSV, virus-like particles (VLPs) containing influenza A–derived hemagglutinin and/or matrix protein 1 or VLPs containing the HIV-1-derived proteins Gag (group antigen) and/or Env (envelope), NK cells mediate vigorous DTH responses after subcutaneous injection of recall antigen, as long as the same antigen is used for vaccination and challenge. Just as with haptens, adoptive-transfer experiments have shown that hepatic but not splenic donor NK cells are required and sufficient to mediate recall responses. Antiviral recall responses by hepatic NK cells are also adaptive in nature, as they are sensitization dependent, long-lived, virus specific and protective. Together these findings establish that at least some NK cells, although they are unable to express RAG proteins, can mediate adaptive immune responses to a variety of viral antigens.

Induction of memory NK cells

Ex vivo exposure to activating cytokines, such as interleukin 12 and interleukin 18, elicits a form of memory in splenic NK cells whereby the primed cells mediate enhanced IFN-γ responses after restimulation by cytokines or by antibody-mediated ligation of activating receptors[27]. Thus, NK cells can acquire certain memory-like properties even without exposure to a specific antigen, similar to the cytokine-driven, antigen-independent 'bystander' response of CD8^{+} T cells[28]. Cytokines are also essential in shaping the antigen-specific effector and memory responses of T cells and B cells[29]; however, the role of cytokines in the induction of antigen-restricted memory NK cells is still unclear.

Experimental results, mainly from CHS studies, are beginning to paint a (still incomplete) picture of the 'career path' taken by memory NK cells (**Fig. 1**). Naive NK cells survey the body and, after ligation of one or more putative antigen receptor(s), 'cognate' NK cells differentiate into 'memory cells' that obtain long-term shelter in the liver. The hepatic memory pool probably dispatches some cells into the blood, maintaining immune surveillance at a low but constant amount. When recall antigen is encountered in the periphery, antigen-specific NK cells accumulate at the site of challenge, presumably after having been released from the liver. These cells then orchestrate local effector responses, such as CHS, DTH or antiviral immunity.

In CHS experiments, haptens are applied to intact skin during sensitization and challenge and are taken up by migratory DCs that transport the material to draining lymph nodes for presentation to recirculating lymphocytes[30] (**Fig. 2a**). Consequently, CHS responses in wild-type and *Rag2*$^{-/-}$ mice are markedly attenuated when the mice are sensitized in the absence of functional L-selectin, a key adhesion receptor for the homing of lymphocytes to peripheral lymph nodes[11,31]. In contrast, CHS responses are not affected by inhibition of L-selectin during the challenge phase when effector cells access the skin[11,31]. Hence, like

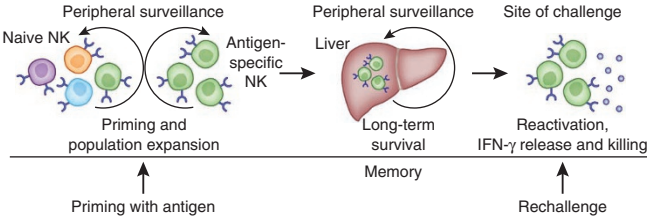


Figure 1 Proposed model for the generation, maintenance and reactivation of NK cell memory. Naive polyclonal NK cells circulate through blood and tissues during peripheral surveillance. Priming of NK cells with a hapten or virus activates antigen-specific NK cells, which proliferate and localize to the liver, where they can persist for many months. During the memory phase, antigen-specific NK cells may be released from the liver to survey peripheral tissues for recall antigen. Challenge with antigen leads to mobilization and reactivation of memory NK cell, which migrate to the site(s) of challenge and mediate effector functions, including the release of proinflammatory cytokines (IFN-γ) and antigen-specific killing.

T cells, hapten-specific NK cells can be primed in peripheral lymph nodes, most probably by migratory DCs[32] (**Fig. 2b**). Likewise, subcutaneous injection of ultraviolet light–inactivated VSV or VLPs generates memory NK cells, presumably after drainage of viral antigens to local lymph nodes, which might be enhanced by coinjection of DCs.

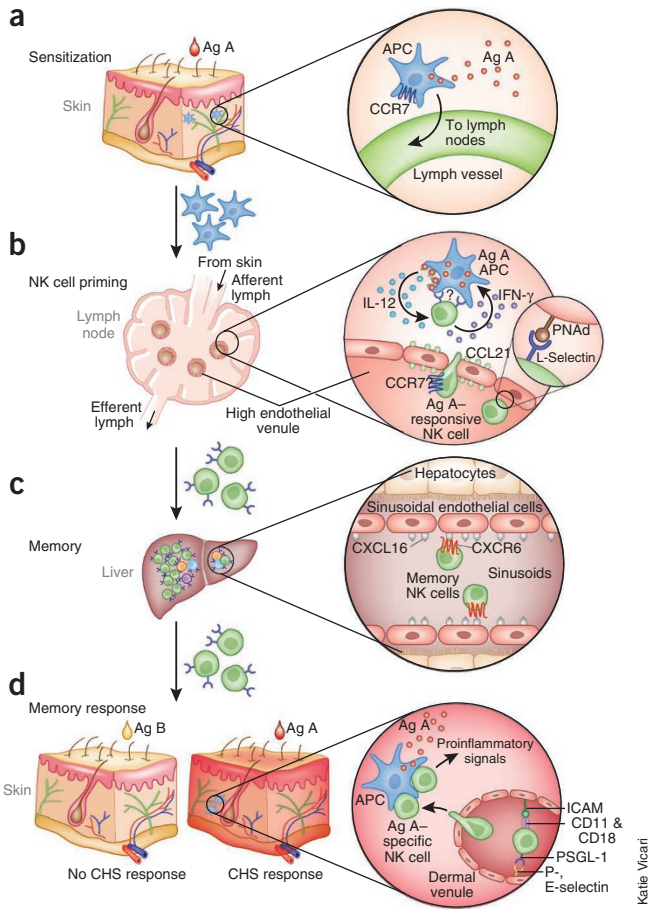
Intravenous administration of hapten-bearing DCs can also sensitize NK cells in *Rag2*$^{-/-}$ mice. As blood-borne DCs home to lymph nodes very poorly[30], it is likely that intravenously injected DCs activate NK cells in other tissues. Indeed, naive T cells can be efficiently sensitized by hapten-bearing DCs in the spleen[31], but it is unclear if this is also true for naive NK cells for antigens other than MCMV. Thus, it is doubtful that the spleen is a major site of memory NK cell differentiation. Indeed, it is possible that naive NK cells are subcategorized a priori into two subsets: one able to acquire memory of a broad range of antigens, which accumulates in the liver and recirculates through lymph nodes; and another with a much more restricted capacity for memory generation, which resides in the spleen.

Figure 2 Key cellular migration events during hapten-induced NK cell–mediated contact hypersensitivity. (**a**) Sensitization of mice by painting of the skin with a hapten (antigen (Ag) A) leads to covalent haptenization of cells and matrix proteins, which are taken up by skin-resident antigen-presenting cells (APC), particularly Langerhans cells, which upregulate CCR7 to access local lymph vessels and travel to a draining lymph node. (**b**) Circulating naive NK cells are recruited to lymph nodes via high endothelial venules, which express peripheral node addressin (PNAd), the ligand for L-selectin (CD62L), which initiates a multistep adhesion cascade[96] that allows NK cells to emigrate into the lymph node. Here, antigen-specific NK cells encounter hapten-presenting skin-derived DCs promoting NK cell activation and differentiation. (**c**) After being activated, the fledgling memory NK cells depart from the lymph node, probably through efferent lymph vessels and the thoracic duct, to reenter the blood stream and home to the liver, where they are retained in the lumen of sinusoids[14]. Persistence of memory NK cells requires NK cell–expressed CXCR6, whose ligand, CXCL16, is constitutively expressed on hepatic sinusoidal endothelial cells. (**d**) Rechallenge of the skin with the same hapten (Ag A) leads to the recruitment and/or retention of antigen-specific memory NK cells in the challenged skin, but rechallenge with a different hapten (Ag B) does not. This process requires that NK cells undergo adhesive interactions with P-selectin and E-selectin, which are constitutively expressed in dermal microvessels[38]. These two selectins mediate rolling interactions that must be followed by activation of β_2 integrins (CD18) to allow firm arrest[11]. ICAM, intercellular adhesion molecule 1 (ligand for integrin $\alpha_L\beta_2$); PSGL-1, P-selectin glycoprotein ligand.

Maintenance of memory NK cells

Once NK cells have been primed in lymph nodes, they return to the blood and migrate to the liver, where they persist as long-lived memory cells[11] (**Fig. 2c**). To reach the circulation, NK cells emigrate from the lymph node parenchyma into efferent lymphatics. This process requires $S1P_5$, a sphingosine 1-phosphate receptor whose expression on NK cells is regulated by the transcription factor T-bet[33]. After entering the liver, blood-borne NK cells presumably respond to recruitment signals that allow them to adhere in hepatic microvessels. NK cells express a diverse repertoire of integrins, selectins, chemokine receptors and other trafficking molecules to access lymphoid and nonlymphoid tissues as well as sites of inflammation[34]. However, the trafficking signals that recruit hapten-primed NK cells to the liver remain uncharacterized.

Of particular interest among the memory NK cell–expressed trafficking molecules is CXCR6, the receptor for CXCL16, a chemokine that occurs as both a secreted polypeptide and a transmembrane glycoprotein. Membrane-bound CXCL16 is present constitutively in liver sinusoids but not in other vascular beds[35]. Accordingly, CXCR6 is rarely found on nonhepatic NK cells, whereas the liver contains roughly equal numbers of CXCR6$^+$ and CXCR6$^-$ NK cells, which represent distinct and stable subsets[14]. The fact that ~50% of hepatic NK cells are CXCR6$^-$ suggests that this receptor is dispensable for the trafficking of NK cells to the liver or their retention within the liver. Indeed, CXCR6 is also expressed on hepatic NKT cells, which do not require CXCR6 to adhere to liver sinusoids but critically depend on CXCR6 signals for long-term survival[35]. Studies of CXCR6-deficient mice, adoptive transfer of purified NK cell subsets into naive hosts and treatment of mice or isolated NK cells with blocking antibodies



indicate that the CXCR6-CXCL16 axis also has a key role in the homeostasis and functional regulation of hepatic memory NK cells[14]. Only CXCR6$^+$ liver NK cells carry transferable memory of haptens and viral antigens, and these cells, unlike the CXCR6$^-$ subset, disappear from the liver less than a day after CXCR6 is blocked, resulting in loss of NK cell memory[14].

In vitro experiments indicate that antigen-specific effector functions mediated by hepatic CXCR6$^+$ NK cells are also regulated by CXCR6. Hapten-sensitized hepatic CXCR6$^-$ NK cells fail to kill hapten-loaded target cells but they do kill MHC class I–deficient targets, which suggests that NK cells require CXCR6 to develop or retain the ability for killing triggered by cognate antigen recognition but not to exert cytotoxicity triggered by 'innate' activating receptors[14]. However, acute inhibition of CXCR6 with antibody *in vitro* enhances the hapten-specific cytotoxicity of CXCR6$^+$ memory NK cells without changing the overall number of antigen-specific cells, whereas activation of CXCR6 by the addition of CXCL16 attenuates cytotoxicity[14]. This indicates that although the CXCR6-CXCL16 pathway is apparently not directly involved in antigen recognition by NK cells, it has a dual role in maintaining the differentiation and/or function of memory NK cells while at the same time preventing full-fledged cytotoxicity. Conceivably, the latter effect might safeguard against excessive hepatotoxicity by liver-resident memory NK cells after systemic antigen exposure.

Immune surveillance by memory NK cells?

Memory NK cells can rapidly respond to peripheral hapten or viral challenge for at least 3–4 months after priming[14]. The hapten-specific hepatic memory population is probably in constant exchange with the blood to patrol peripheral tissues for recall antigen. It should be noted, however, that CXCR6$^+$ NK cells are rare (<5% of NK1.1$^+$ cells) in mouse peripheral blood, which could reflect infrequent release of NK cells from the liver and/or rapid recruitment of released cells into tissues. Although it is also possible that NK cells downregulate CXCR6 when they leave the liver, we do not favor this idea because CXCR6$^+$ NK cells are very stable even after long-term adoptive transfer[14]. In support of the idea that NK cells may constantly leave the circulation, at least in humans, NK cells have been detected in skin-draining lymph fluid from healthy volunteers and patients with contact dermatitis, which suggests that they are recruited from blood to the skin during the steady state and inflammatory disease and return to the blood via draining lymphatics[36]. Although it is unknown whether these recirculating NK cells carry memory of contact sensitizers in humans, the failure to detect such rare cells in patient blood[37] does not provide conclusive evidence of their absence.

Recall responses by memory NK cells

Peripheral antigen challenge of sensitized mice and humans by epidermal painting with contact sensitizers elicits the accumulation of NK cells in the exposed skin[11,14,37] (**Fig. 2d**). This infiltration by antigen-specific NK cells in mouse CHS may reflect, at least in part, antigen-driven proliferation *in situ*. However, the recruitment of (presumably) liver-derived memory NK cells from the blood is a critical step, as CHS responses depend on the ability of NK cells to adhere to P-selectin and E-selectin in dermal microvessels[11,38]. Moreover, memory NK cells exert effector activity not only in the skin but also in many other tissues[11] and sites of exposure to viral antigens[14]. Mature DCs interact with NK cells in inflamed tissues[39] and express CXCL16 (ref. 40), which might supply a survival signal to CXCR6$^+$ memory NK cells in inflamed or infected tissues. Of note, CXCL16 expression is enhanced by IFN-γ[41], which is secreted by hapten-sensitized NK cells after restimulation (S.P. and U.H.v.A., unpublished data). Thus, peripheral encounters with antigen may allow memory NK cells to make a 'home away from home' after their departure from the liver. How such exposure triggers the mobilization of hepatic memory NK cells is unclear at present, although blood-borne signals may encourage their detachment from sinusoidal endothelium.

Once NK cells have emigrated into an effector site, they must detect and respond to the local antigen challenge. Although the molecular mechanisms that confer antigen specificity remain a mystery, haptens and viruses cause nonspecific tissue injury that results in innate 'distress signals' that can be detected by activating NK cell receptors[8]. One such sensor is NKG2D, which promotes proinflammatory effector responses by NK cells when ligated by its MHC class I–related counter-receptors[42]. This pathway also contributes to NK cell–dependent CHS, as antibody inhibition of NKG2D in sensitized *Rag2*$^{-/-}$ mice suppresses hapten-induced CHS[11].

Table 1 Role of CXCR6–CXCL16 in murine models of disease

Disease model	Organ	Role of CXCR6 and/or CXCL16	Ref
Diabetes	Islet cells	Identification of S129P substitution in CXCL16 by SNP analysis of the *Idd4* locus of NOD and NOR mice	55
Atherosclerosis	Cardiovascular system	Acceleration of atherosclerosis in CXCL16-deficient *Ldlr*$^{-/-}$ mice; less atherosclerosis in CXCR6-deficient *Apoe*$^{-/-}$ mice fed a Western diet	60,97
Allergic asthma	Lung	Higher CXCR6 expression on T cells, NKT cells and NK cells after allergen challenge	98
Graft-versus-host disease: bone marrow transplantation	Lung, liver, GI tract	Higher expression of CXCL16 and CXCR6 in GI tract and liver; higher CXCR6 expression in lungs	64
Heart transplantation	Heart	Less NKT cell accumulation and more transplant rejection after blockade of CXCR6-CXCL16 pathway	63
CNS and cortical injury	Brain	Requirement for CXCR6 expression for the infiltration of lymphocyte to sites of cortical injury	54
		IFN-γ$^+$CXCR6$^+$ MBP-reactive T cells; correlation of CXCR6 expression with effector memory phenotype	41
Nephritis	Glomeruli	Upregulation of CXCR6 and CXCL16 mRNA in glomeruli of MRL-lpr mice treated with prednisolone	58
		Attenuation of glomerulonephritis associated with antibody to glomerular basement membrane after inhibition of CXCL16	56
Rheumatoid arthritis	Synovial fluid and draining lymph nodes	More soluble CXCL16 in synovial fluid; 30–50% of infiltrating leukocytes express CXCR6	59

SNP, single-nucleotide polymorphism; *Idd4*, insulin-dependent diabetes susceptibility 4; NOD, nonobese diabetic; NOR, nonobese resistant; *Ldlr*, gene encoding the low-density lipoprotein receptor; *Apoe*, gene encoding apolipoprotein E; GI, gastrointestinal; CNS, central nervous system; MBP, myelin basic protein; MRL-lpr, MRL mice with the lymphoproliferation mutation.

NK cells can profoundly influence the quality and magnitude of T cell and B cell responses by activating or killing antigen-presenting cells and regulatory T cells[43,44], by modulating the generation and effector functions of cytotoxic T cells[45], by skewing helper T cell polarization[46], and by enhancing B cell activation and isotype switching[47]. NK cells can therefore augment or ameliorate autoimmune diseases[48]. In humans, predisposition to rheumatoid arthritis[49], psoriatic arthritis[50], scleroderma[51] and psoriasis[52] has been linked to certain NK cell subsets. Conversely, CXCR6 and CXCL16 have been linked to many diseases in mice (**Table 1**) and humans (**Table 2**), including autoimmunity[53–59], inflammatory disorders[60–62], graft-versus-host disease[63,64], cancer[65–69] and HIV-AIDS[70–73]. Although it is intriguing in this context that mouse NK cells depend on CXCR6 to develop specific memory of HIV-1, it is unknown whether the contribution of human NK cells to the control of HIV-1 infection or any other condition involves the CXCR6-CXCL16 pathway.

How do memory NK cells recognize 'their' antigen?

Antigen-specific NK cell memory has been documented for three distinct haptens (DNFB, oxazolone and picryl chloride[11,14]) and at least four viruses (MCMV[13], VSV, influenza and HIV-1 (ref. 14)). In particular, CHS responses are remarkably antigen restricted in mice, and there is essentially no cross-reactivity between DNFB and picryl chloride, which are structurally similar. How do NK cells detect, remember and distinguish these diverse antigens?

NK cells survey their environment through the use of a finite number of germline-encoded receptors that detect either inhibitory signals or activating signals[8,74], as well as cytokines and chemokines. So far, the mechanism of antigen recognition and memory is understood only for MCMV[13]. However, there are notable differences between MCMV and other viral infection models in mice. MCMV-reactive NK cells are not restricted to the liver[13], and the cognate MCMV receptor Ly49H[75] is absent from most mouse strains other than C57BL/6 (ref. 76). In contrast, NK cell memory of VSV, influenza and HIV-1 is concentrated in the liver and is inducible in diverse mouse strains, including C57BL/6 and BALB/c, which express distinct MHC haplotypes and members of the Ly49 lectin-like receptor family[14].

The Ly49 family in mice and the killer cell immunoglobulin-like receptor (KIR) family in humans[74] are examples of activating and inhibitory receptors expressed by NK cells. NK cells express a random selection of one or more of these, in addition to activating natural cytotoxicity receptors, NKG2D and others. Several members of the Ly49 and KIR families recognize MHC class I, and some have evolved to detect virus-encoded gene products[75] are examples of activating and inhibitory receptors expressed by NK cells. No specific NK receptor has been identified for VSV, but influenza hemagglutinin is a ligand for Nkp46, an activating receptor on mouse and human NK cells[77,78]. However, NK cells develop specific protective memory of influenza virus after sensitization with VLPs containing only influenza matrix protein 1 without hemagglutinin, which indicates that recognition of hemagglutinin by Nkp46 is not required for the generation of memory[14]. Although neither VSV nor influenza is endemic in mice, both can cause lethal infection in rodents, so it is possible that other, as-yet-unidentified germline-encoded receptors may exert a function similar to that of Ly49H in LCMV infection. However, it is difficult to envision how mice could have evolved specific receptors for haptens or HIV-1, which cannot infect mice. Indeed, extensive flow cytometry and microarray analysis of DNFB- and oxazolone-sensitized NK cells has failed to detect substantial changes in the frequency or expression of Ly49 family members or other known pattern-recognition receptors on NK cells[11] (S.P. and U.H.v.A., unpublished data).

As described above, NK cell licensing occurs in mice during NK cell development and is believed to ensure that only NK cells able to engage self MHC class I with one or more inhibitory Ly49 receptor(s) are fully responsive to certain activating stimuli[79]. DNFB- and oxazolone-specific NK cell memory in C57BL/10 mice is

Table 2 Role of CXCR6–CXCL16 in human disease

Disease	Organ or cell type	Role of CXCR6 and/or CXCL16	Ref
Diabetes	T cells	Lower CXCR6 expression on $CD4^+$ and $CD8^+$ T cells in children with type 1 diabetes	53
	BM-MSCs	BM-MSCs isolated from pancreatic islets express CXCR6	57
Crohn's disease	Colon	Higher concentration of CXCL16 in serum and colon from patients with Crohn's disease	99
Prostate cancer	Prostate	More invasive growth and angiogenesis in tumors with high CXCR6 expression	68
Carcinoma cell lines and primary carcinoma	Breast, colon, pancreas	More growth after ligation of CXCR6; less growth after ligation of transmembrane ligation of expressed CXCL16	69
		Upregulation of CXCL16 by carcinoma cells after ionizing radiation	100
Renal cell carcinoma	Kidney	Inverse correlation between CXCL16 expression on renal cell carcinoma and tumor stage	67
Melanoma	Skin	Expression of CXCR6 in melanoma and melanoma metastases	66
HIV-AIDS	Immune system	CXCR6 is a coreceptor for HIV-1 and HIV-2	72,101
		Polymorphism in the 3′ untranslated region of *CXCR6* correlates with long-term nonprogression to AIDS	71
		More time from initial AIDS diagnosis to pneumonia-related death in African-American users of injected drugs who are homozygous for the *CXCR6*-3K polymorphism	73
Chronic inflammation	Liver (infected with HCV)	Higher CXCL16 expression at HCV-hepatic interphase; more infiltration and retention of $CXCR6^+$ lymphocytes	102
Chronic inflammation	Liver (GvH hepatitis)	More recruitment of $CXCR6^+$ CD8 T cells to inflamed liver	103
Psoriasis	Skin	Overexpression of CXCL16 in psoriatic skin	104
Juvenile idiopathic arthritis	Inflamed joints	Expression of CXCR6 and CXCL16 by synoviocytes, macrophages and endothelial cells; $CXCR6^+$ infiltrating T cells	105
Rheumatoid arthritis	Synovial fluid and draining lymph nodes	Enhanced production of CXCL16 in synovia and synovial macrophages from patients with rheumatoid arthritis leads to recruitment of $CD8^+$ memory T cells	106,107

BM-MSC, bone marrow mesenchymal stem cell; HCV, hepatitis C virus; GvH, graft-versus-host.

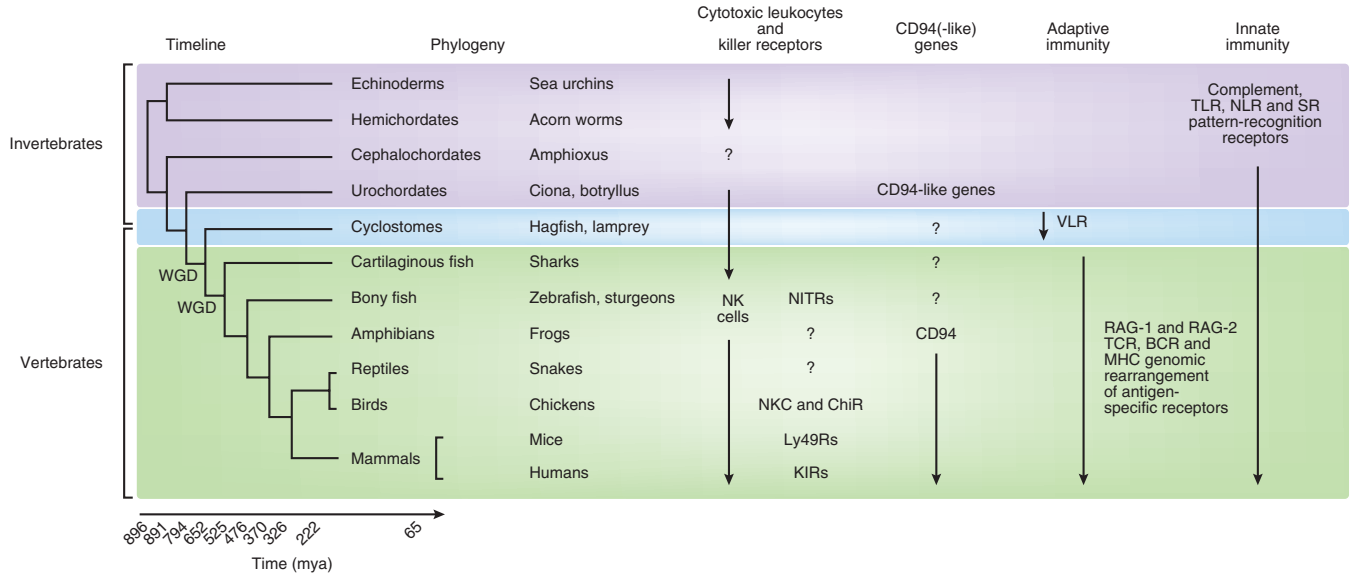


Figure 3 Overview of innate and adaptive immunity and cytotoxic leukocytes in deuterostomes. The divergence of deuterostomes is presented here, including events during phylogeny at which cellular or molecular features of relevance for adaptive immunity and/or NK cells first emerged. Invertebrates and vertebrates rely on the innate immune system, which includes complement, Toll-like receptors (TLR), Nod-like receptors (NLR) and scavenger receptors (SR) that mediate pattern recognition to respond to tissue damage and infection. Additionally, vertebrates mount clonal adaptive immune responses. Although cyclostomes make use of cytidine deaminase–dependent variable lymphocyte receptors (VLR), jawed vertebrates express T cell antigen receptors (TCR) and B cell antigen receptors (BCR) dependent on the products of *Rag1* and *Rag2*. Natural killing, defined as cell contact–dependent killing that requires no prior sensitization, has been described in echinoderms and hemichordates, in which phagocytic amebocytes or tunicate hemocytes mediate spontaneous cytotoxicity. Orthologs of the NK cell–associated lectin-like receptor CD94 have been identified in urochordates, such as ciona and botryllus, which spontaneously lyse allogeneic cells. Some cells derived from hagfish and sea lamprey, both jawless vertebrates (agnathans), are also capable of spontaneous lysis of allogeneic cells, although neither NK cells nor orthologs of NK cell–associated genes have been identified in agnathans. The genomes of jawed vertebrates encode RAG-1, RAG-2, T cell and B cell antigen receptors, MHC, CD94 and a variety of receptors expressed by natural cytotoxic cells, such as NITRs (novel immune-type receptors) in fish, ChiR (chicken immunoglobulin-like receptors) in chickens, the KIR family in humans and the Ly49 receptors in mice. WGD, whole-genome duplication; mya, million years ago.

concentrated in a hepatic NK cell subset that expresses Ly49C and/or Ly49I, which recognize H-2D^b in that strain; this suggests that hapten-specific memory NK cells must also be licensed[11]. Although the engagement of self MHC class I is thought to send an inhibitory signal to NK cells, hapten-sensitized hepatic but not splenic NK cells efficiently kill haptenated B cells with normal expression of MHC class I[14]. In contrast, both splenic and hepatic NK cells kill MHC class I–deficient targets, which indicates that the killing of haptenated MHC class I–sufficient B cells is not due to masking of MHC class I by covalently bound haptens. Instead, the pathway triggered in hepatic memory NK cells by cognate antigen apparently overrides inhibitory signals from MHC class I[14].

So far, antigen-specific NK cell memory has been documented only in mice. However, many observations from studies of humans and non-human primates are worth noting. Hantavirus-infected patients have an expanded population of multifunctional NKG2C$^+$ NK cells as late as 60 days after infection[80], which suggests that human NK cells may have the capacity for long-term persistence. Whether the expanded NK cell subset shows evidence of clonality and/or viral specificity remains undetermined. Subset-restricted NK cell–mediated antiviral immunity, albeit not immunological memory, has been demonstrated in patients with HIV-1 infection in which coexpression of certain KIR and HLA-I alleles correlates with disease progression[81]. Similar observations have also been reported for hepatitis C virus infection[82]. Immune responses in macaques infected with simian immunodeficiency virus also involve distinct NK cell subsets, which seem to function in an organ-specific manner[83]. A multiple-cohort genome-wide association study of patients infected with HIV-1 long-term who did not develop AIDS despite chronic viremia has found that the rs2234358 polymorphism in *CXCR6* is strongly associated with long-term nonprogression to AIDS[71,73]. CXCR6 is expressed on a subset of human NK cells in peripheral blood[84], but its distribution in human liver is unknown. Further work is needed to establish whether and how CXCR6 and NK cells are linked in conferring resistance to HIV-1 infection.

NK cells in evolution

Does NK cell memory represent an ancient defense mechanism against certain types of infections or is it a recent invention, possibly unique to mice? The answers to this probably must await the elucidation of the molecular underpinnings of NK cell memory. Arguably the best-studied immune function of NK cells relies on members of the Ly49 family and KIR family in mice and humans, respectively. Although these two receptor families serve the same functions, they are structurally unrelated and their genes are located on different chromosomes[85], which suggests that they and their immunological functions evolved rapidly after the divergence of rodents and primates ~65 million years ago. However, NK cell–like cells are also found in primitive vertebrates, including bony fish (**Fig. 3**), and some NK cells in mouse thymus do not express Ly49 receptors, which suggests that they may have Ly49-independent biological functions[86].

It is unclear when NK cells first evolved, mainly because their identification in lower animals depends on the criteria used to distinguish NK cells from other cells of the immune response. One defining feature is the ability to perform contact-dependent killing of target cells without requiring priming. Cells with such ability can be found in metazoans[87]. Marine sponges and corals

use cytotoxic cells to avoid fusion with one another[88]; however, it is not known if these processes involve immunoglobulin or lectin-like receptors, whose expression is central to NK cell function in jawed vertebrates (gnathostomes). Whether NK cells predate or depend on recognition of MHC complexes is also not clear. As discussed above, mammalian NK cell receptors for MHC class I developed relatively late, but another quintessential NK cell marker, the lectin-like receptor CD94, is at least 400 million years old[89], and a CD94-like molecule has even been identified in a tunicate, *Ciona intestinalis*, whose last common ancestor with vertebrates lived ~750 million years ago[90].

The T cell– and B cell–based adaptive immune system is believed to have originated ~500 million years ago when two rounds of genome-wide duplication led to the creation of MHC-paralogous regions and the NK receptor gene complex[91]. Recombination-activating genes have been isolated from all gnathostomes studied[85]; however, adaptive immune responses and RAG-independent production of clonal lymphocytes have also been described in two jawless vertebrates (agnathans), the sea lamprey and hagfish[92]. Agnathans are limited to the primordial MHC and do not express immunoglobulin superfamily immune receptors or RAG proteins but use cytidine deaminases to assemble genes encoding variable lymphocyte receptors, which are glycosylphosphatidylinositol-anchored antigen receptors composed of leucine-rich repeats. This process can potentially generate up to 1×10^{14} different antigen-recognition receptors[92] that mediate adaptive, clonal immune responses, including immunity to infection, allograft rejection and DTH. Orthologs of several genes important for adaptive immune responses have been identified in lamprey[93,94], and many signaling molecules that control mammalian lymphocyte activation and/or effector function are also present in these animals[95]. Agnathans are thought to have evolved before gnathostomes and gave rise to the latter; however, it is unknown whether the variable lymphocyte receptor system was already in place when jawed and jawless vertebrates diverged.

Concluding remarks

Since the first report of NK cell–mediated adaptive immunity to haptens[11], evidence of NK cell memory has continued to accumulate and expanded to include protective antiviral immunity[12,13,27]. However, many questions remain unanswered. Arguably, identification of the molecular mechanisms by which NK cells generate what seems to be exquisite specificity for highly diverse antigens (other than MCMV) must be given priority. Work on these questions will require new molecular and genetic tools whose absence has hampered progress so far. As there is no method at present to generate antigen-specific memory NK cells *in vitro*, investigators must rely on *in vivo*–induced memory cells that can be obtained only by isolation of the relatively few NK cells residing in the liver, ideally from RAG-deficient donors to avoid contamination by other lymphocytes. Among these NK cells only a small fraction is specific for any given antigen, and there are no markers at present with which to identify this subset. The fact that memory NK cells seem to be extremely rare in peripheral blood also complicates the exploration of NK cell memory in humans, as it is difficult to obtain fresh liver tissue from humans. Nonetheless, it will be critical to determine whether NK cell memory arises in mammals other than mice. If humans are found to have an NK cell–based adaptive immune system, it will be important to understand how this system contributes to diseases and how it may be clinically exploited or manipulated to prevent or treat human suffering.

ACKNOWLEDGMENTS

Supported by the Cancer Research Institute (S.P.), the Ragon Institute of MGH, MIT and Harvard (S.P. and U.H.v.A.) and the US National Institutes of Health (AI069259, AI072252 and AI1078897 to U.H.v.A.).

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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1. Bassing, C.H., Swat, W. & Alt, F.W. The mechanism and regulation of chromosomal V(D)J recombination. *Cell* **109**, S45–S55 (2002).
2. Phanuphak, P., Moorhead, J.W. & Claman, H.N. Tolerance and contact sensitivity to DNFB in mice. II. Specific in vitro stimulation with a hapten, 2,4-dinitrobenzene sulfonic acid (DNB-SO3Na). *J. Immunol.* **112**, 849–851 (1974).
3. Phanuphak, P., Moorhead, J.W. & Claman, H.N. Tolerance and contact sensitivity to DNFB in mice. I. In vivo detection by ear swelling and correlation with in vitro cell stimulation. *J. Immunol.* **112**, 115–123 (1974).
4. Crowle, A.J. Delayed hypersensitivity in mice; its detection by skin tests and its passive transfer. *Science* **130**, 159–160 (1959).
5. Quan, F.S., Huang, C., Compans, R.W. & Kang, S.M. Virus-like particle vaccine induces protective immunity against homologous and heterologous strains of influenza virus. *J. Virol.* **81**, 3514–3524 (2007).
6. Herberman, R.B., Nunn, M.E. & Lavrin, D.H. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. I. Distribution of reactivity and specificity. *Int. J. Cancer* **16**, 216–229 (1975).
7. Herberman, R.B., Nunn, M.E., Holden, H.T. & Lavrin, D.H. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. II. Characterization of effector cells. *Int. J. Cancer* **16**, 230–239 (1975).
8. Lanier, L.L. NK cell recognition. *Annu. Rev. Immunol.* **23**, 225–274 (2005).
9. Walzer, T., Dalod, M., Robbins, S.H., Zitvogel, L. & Vivier, E. Natural-killer cells and dendritic cells: "l'union fait la force". *Blood* **106**, 2252–2258 (2005).
10. Orange, J.S. Human natural killer cell deficiencies. *Curr. Opin. Allergy Clin. Immunol.* **6**, 399–409 (2006).
11. O'Leary, J.G., Goodarzi, M., Drayton, D.L. & von Andrian, U.H. T cell– and B cell–independent adaptive immunity mediated by natural killer cells. *Nat. Immunol.* **7**, 507–516 (2006).
12. Paust, S., Senman, B. & von Andrian, U.H. Adaptive immune responses mediated by natural killer cells. *Immunol. Rev.* **235**, 286–296 (2010).
13. Sun, J.C., Beilke, J.N. & Lanier, L.L. Adaptive immune features of natural killer cells. *Nature* **457**, 557–561 (2009).
14. Paust, S. *et al.* Critical role for the chemokine receptor CXCR6 in NK cell-mediated antigen-specific memory of haptens and viruses. *Nat. Immunol.* **11**, 1127–1135 (2010).
15. Askenase, P.W. Yes T cells, but three different T cells (αβ, γδ and NK T cells), and also B-1 cells mediate contact sensitivity. *Clin. Exp. Immunol.* **125**, 345–350 (2001).
16. Gorbachev, A.V. & Fairchild, R.L. Induction and regulation of T-cell priming for contact hypersensitivity. *Crit. Rev. Immunol.* **21**, 451–472 (2001).
17. Boehncke, W.H. *et al.* Leukocyte extravasation as a target for anti-inflammatory therapy—which molecule to choose? *Exp. Dermatol.* **14**, 70–80 (2005).
18. Cao, X. *et al.* Defective lymphoid development in mice lacking expression of the common cytokine receptor gamma chain. *Immunity* **2**, 223–238 (1995).
19. DiSanto, J.P., Muller, W., Guy-Grand, D., Fischer, A. & Rajewsky, K. Lymphoid development in mice with a targeted deletion of the interleukin 2 receptor gamma chain. *Proc. Natl. Acad. Sci. USA* **92**, 377–381 (1995).
20. MacDougall, J.R., Croy, B.A., Chapeau, C. & Clark, D.A. Demonstration of a splenic cytotoxic effector cell in mice of genotype SCID/SCID.BG/BG. *Cell. Immunol.* **130**, 106–117 (1990).
21. Vermijlen, D. *et al.* High-density oligonucleotide array analysis reveals extensive differences between freshly isolated blood and hepatic natural killer cells. *Eur. J. Immunol.* **34**, 2529–2540 (2004).
22. Ochi, M. *et al.* Liver NK cells expressing TRAIL are toxic against self hepatocytes in mice. *Hepatology* **39**, 1321–1331 (2004).
23. Ishiyama, K. *et al.* Difference in cytotoxicity against hepatocellular carcinoma between liver and periphery natural killer cells in humans. *Hepatology* **43**, 362–372 (2006).
24. Kim, S. *et al.* Licensing of natural killer cells by host major histocompatibility complex class I molecules. *Nature* **436**, 709–713 (2005).
25. Smith, H.R. *et al.* Recognition of a virus-encoded ligand by a natural killer cell activation receptor. *Proc. Natl. Acad. Sci. USA* **99**, 8826–8831 (2002).
26. Arase, H., Mocarski, E.S., Campbell, A.E., Hill, A.B. & Lanier, L.L. Direct recognition of cytomegalovirus by activating and inhibitory NK cell receptors. *Science* **296**, 1323–1326 (2002).
27. Cooper, M.A. *et al.* Cytokine-induced memory-like natural killer cells. *Proc. Natl. Acad. Sci. USA* **106**, 1915–1919 (2009).

28. Zhang, X., Sun, S., Hwang, I., Tough, D.F. & Sprent, J. Potent and selective stimulation of memory-phenotype CD8+ T cells in vivo by IL-15. *Immunity* **8**, 591–599 (1998).
29. Sallusto, F. & Lanzavecchia, A. Heterogeneity of CD4+ memory T cells: Functional modules for tailored immunity. *Eur. J. Immunol.* **39**, 2076–2082 (2009).
30. Alvarez, D., Vollmann, E.H. & von Andrian, U.H. Mechanisms and consequences of dendritic cell migration. *Immunity* **29**, 325–342 (2008).
31. Catalina, M.D. *et al.* The route of antigen entry determines the requirement for L-selectin during immune responses. *J. Exp. Med.* **184**, 2341–2351 (1996).
32. Bajenoff, M. *et al.* Natural killer cell behavior in lymph nodes revealed by static and real-time imaging. *J. Exp. Med.* **203**, 619–631 (2006).
33. Jenne, C.N. *et al.* T-bet-dependent S1P5 expression in NK cells promotes egress from lymph nodes and bone marrow. *J. Exp. Med.* **206**, 2469–2481 (2009).
34. Morris, M.A. & Ley, K. Trafficking of natural killer cells. *Curr. Mol. Med.* **4**, 431–438 (2004).
35. Geissmann, F. *et al.* Intravascular immune surveillance by CXCR6+ NKT cells patrolling liver sinusoids. *PLoS Biol.* **3**, e113 (2005).
36. Hunger, R.E., Yawalkar, N., Braathen, L.R. & Brand, C.U. The HECA-452 epitope is highly expressed on lymph cells derived from human skin. *Br. J. Dermatol.* **141**, 565–569 (1999).
37. Carbone, T. *et al.* CD56^{high}CD16^{-}CD62L^{-} NK cells accumulate in allergic contact dermatitis and contribute to the expression of allergic responses. *J. Immunol.* **184**, 1102–1110 (2010).
38. Weninger, W. *et al.* Specialized contributions by α(1,3)-fucosyltransferase-IV and FucT-VII during leukocyte rolling in dermal microvessels. *Immunity* **12**, 665–676 (2000).
39. Buentke, E. *et al.* Natural killer and dendritic cell contact in lesional atopic dermatitis skin—Malassezia-influenced cell interaction. *J. Invest. Dermatol.* **119**, 850–857 (2002).
40. van der Voort, R. *et al.* An alternatively spliced CXCL16 isoform expressed by dendritic cells is a secreted chemoattractant for CXCR6+ cells. *J. Leukoc. Biol.* **87**, 1029–1039 (2010).
41. Calabresi, P.A., Yun, S.H., Allie, R. & Whartenby, K.A. Chemokine receptor expression on MBP-reactive T cells: CXCR6 is a marker of IFNγ-producing effector cells. *J. Neuroimmunol.* **127**, 96–105 (2002).
42. Raulet, D.H. Roles of the NKG2D immunoreceptor and its ligands. *Nat. Rev. Immunol.* **3**, 781–790 (2003).
43. Ferlazzo, G. *et al.* The interaction between NK cells and dendritic cells in bacterial infections results in rapid induction of NK cell activation and in the lysis of uninfected dendritic cells. *Eur. J. Immunol.* **33**, 306–313 (2003).
44. Jinushi, M. *et al.* Natural killer cell and hepatic cell interaction via NKG2A leads to dendritic cell-mediated induction of CD4 CD25 T cells with PD-1-dependent regulatory activities. *Immunology* **120**, 73–82 (2007).
45. Nocentini, G., Ronchetti, S., Cuzzocrea, S. & Riccardi, C. GITR/GITRL: more than an effector T cell co-stimulatory system. *Eur. J. Immunol.* **37**, 1165–1169 (2007).
46. Martin-Fontecha, A. *et al.* Induced recruitment of NK cells to lymph nodes provides IFN-γ for T_H1 priming. *Nat. Immunol.* **5**, 1260–1265 (2004).
47. Gao, N., Dang, T. & Yuan, D. IFN-gamma-dependent and -independent initiation of switch recombination by NK cells. *J. Immunol.* **167**, 2011–2018 (2001).
48. Lunemann, A., Lunemann, J.D. & Munz, C. Regulatory NK-cell functions in inflammation and autoimmunity. *Mol. Med.* **15**, 352–358 (2009).
49. Namekawa, T. *et al.* Killer cell activating receptors function as costimulatory molecules on CD4+CD28^{null} T cells clonally expanded in rheumatoid arthritis. *J. Immunol.* **165**, 1138–1145 (2000).
50. Martin, M.P. *et al.* Cutting edge: susceptibility to psoriatic arthritis: influence of activating killer Ig-like receptor genes in the absence of specific HLA-C alleles. *J. Immunol.* **169**, 2818–2822 (2002).
51. Momot, T. *et al.* Association of killer cell immunoglobulin-like receptors with scleroderma. *Arthritis Rheum.* **50**, 1561–1565 (2004).
52. Suzuki, Y. *et al.* Genetic polymorphisms of killer cell immunoglobulin-like receptors are associated with susceptibility to psoriasis vulgaris. *J. Invest. Dermatol.* **122**, 1133–1136 (2004).
53. Hedman, M., Faresjo, M., Axelsson, S., Ludvigsson, J. & Casas, R. Impaired CD4 and CD8 T cell phenotype and reduced chemokine secretion in recent-onset type 1 diabetic children. *Clin. Exp. Immunol.* **153**, 360–368 (2008).
54. Kim, J.V. *et al.* Two-photon laser scanning microscopy imaging of intact spinal cord and cerebral cortex reveals requirement for CXCR6 and neuroinflammation in immune cell infiltration of cortical injury sites. *J. Immunol. Methods* **352**, 89–100 (2009).
55. Ivakine, E.A. *et al.* Molecular genetic analysis of the Idd4 locus implicates the IFN response in type 1 diabetes susceptibility in nonobese diabetic mice. *J. Immunol.* **176**, 2976–2990 (2006).
56. Garcia, G.E. *et al.* Inhibition of CXCL16 attenuates inflammatory and progressive phases of anti-glomerular basement membrane antibody-associated glomerulonephritis. *Am. J. Pathol.* **170**, 1485–1496 (2007).
57. Sordi, V. *et al.* Bone marrow mesenchymal stem cells express a restricted set of functionally active chemokine receptors capable of promoting migration to pancreatic islets. *Blood* **106**, 419–427 (2005).
58. Teramoto, K. *et al.* Microarray analysis of glomerular gene expression in murine lupus nephritis. *J. Pharmacol. Sci.* **106**, 56–67 (2008).
59. Ruth, J.H. *et al.* CXCL16-mediated cell recruitment to rheumatoid arthritis synovial tissue and murine lymph nodes is dependent upon the MAPK pathway. *Arthritis Rheum.* **54**, 765–778 (2006).
60. Aslanian, A.M. & Charo, I.F. Targeted disruption of the scavenger receptor and chemokine CXCL16 accelerates atherosclerosis. *Circulation* **114**, 583–590 (2006).
61. Galkina, E. & Ley, K. Leukocyte influx in atherosclerosis. *Curr. Drug Targets* **8**, 1239–1248 (2007).
62. Wuttge, D.M. *et al.* CXCL16/SR-PSOX is an interferon-γ-regulated chemokine and scavenger receptor expressed in atherosclerotic lesions. *Arterioscler. Thromb. Vasc. Biol.* **24**, 750–755 (2004).
63. Jiang, X. *et al.* Cutting edge: critical role of CXCL16/CXCR6 in NKT cell trafficking in allograft tolerance. *J. Immunol.* **175**, 2051–2055 (2005).
64. Bouazzaoui, A. *et al.* Chemokine and chemokine receptor expression analysis in target organs of acute graft-versus-host disease. *Genes Immun.* **10**, 687–701 (2009).
65. Matsumura, K. *et al.* Radioimmunoscintigraphy of pancreatic cancer in tumor-bearing athymic nude mice using ^{99m}technetium-labeled anti-KL-6/MUC1 antibody. *Radiat. Med.* **26**, 133–139 (2008).
66. Seidl, H. *et al.* Profiles of chemokine receptors in melanocytic lesions: de novo expression of CXCR6 in melanoma. *Hum. Pathol.* **38**, 768–780 (2007).
67. Gutwein, P. *et al.* Tumoural CXCL16 expression is a novel prognostic marker of longer survival times in renal cell cancer patients. *Eur. J. Cancer* **45**, 478–489 (2009).
68. Wang, J., Lu, Y., Koch, A.E., Zhang, J. & Taichman, R.S. CXCR6 induces prostate cancer progression by the AKT/mammalian target of rapamycin signaling pathway. *Cancer Res.* **68**, 10367–10376 (2008).
69. Meijer, J. *et al.* The chemokine receptor CXCR6 and its ligand CXCL16 are expressed in carcinomas and inhibit proliferation. *Cancer Res.* **68**, 4701–4708 (2008).
70. Liao, F. *et al.* STRL33, A novel chemokine receptor-like protein, functions as a fusion cofactor for both macrophage-tropic and T cell line-tropic HIV-1. *J. Exp. Med.* **185**, 2015–2023 (1997).
71. Limou, S. *et al.* Multiple-cohort genetic association study reveals CXCR6 as a new chemokine receptor involved in long-term nonprogression to AIDS. *J. Infect. Dis.* **202**, 908–915 (2010).
72. Blaak, H. *et al.* CCR5, GPR15, and CXCR6 are major coreceptors of human immunodeficiency virus type 2 variants isolated from individuals with and without plasma viremia. *J. Virol.* **79**, 1686–1700 (2005).
73. Duggal, P. *et al.* Genetic influence of CXCR6 chemokine receptor alleles on PCP-mediated AIDS progression among African Americans. *Genes Immun.* **4**, 245–250 (2003).
74. Lanier, L.L. Up on the tightrope: natural killer cell activation and inhibition. *Nat. Immunol.* **9**, 495–502 (2008).
75. Lanier, L.L. Evolutionary struggles between NK cells and viruses. *Nat. Rev. Immunol.* **8**, 259–268 (2008).
76. Scalzo, A.A., Manzur, M., Forbes, C.A., Brown, M.G. & Shellam, G.R. NK gene complex haplotype variability and host resistance alleles to murine cytomegalovirus in wild mouse populations. *Immunol. Cell Biol.* **83**, 144–149 (2005).
77. Gazit, R. *et al.* Lethal influenza infection in the absence of the natural killer cell receptor gene Ncr1. *Nat. Immunol.* **7**, 517–523 (2006).
78. Mandelboim, O. *et al.* Recognition of haemagglutinins on virus-infected cells by NKp46 activates lysis by human NK cells. *Nature* **409**, 1055–1060 (2001).
79. Yokoyama, W.M. & Kim, S. Licensing of natural killer cells by self-major histocompatibility complex class I. *Immunol. Rev.* **214**, 143–154 (2006).
80. Bjorkstrom, N.K. *et al.* Rapid expansion and long-term persistence of elevated NK cell numbers in humans infected with hantavirus. *J. Exp. Med* **208**, 13–21 (2011).
81. Martin, M.P. *et al.* Epistatic interaction between KIR3DS1 and HLA-B delays the progression to AIDS. *Nat. Genet.* **31**, 429–434 (2002).
82. Khakoo, S.I. *et al.* HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. *Science* **305**, 872–874 (2004).
83. Reeves, R.K. *et al.* CD16- natural killer cells: enrichment in mucosal and secondary lymphoid tissues and altered function during chronic SIV infection. *Blood* **115**, 4439–4446 (2011).
84. Berahovich, R.D., Lai, N.L., Wei, Z., Lanier, L.L. & Schall, T.J. Evidence for NK cell subsets based on chemokine receptor expression. *J. Immunol.* **177**, 7833–7840 (2006).
85. Cooper, M.D. & Alder, M.N. The evolution of adaptive immune systems. *Cell* **124**, 815–822 (2006).
86. Vosshenrich, C.A. *et al.* A thymic pathway of mouse natural killer cell development characterized by expression of GATA-3 and CD127. *Nat. Immunol.* **7**, 1217–1224 (2006).
87. Fernandez-Busquets, X. & Burger, M.M. The main protein of the aggregation factor responsible for species-specific cell adhesion in the marine sponge *Microciona prolifera* is highly polymorphic. *J. Biol. Chem.* **272**, 27839–27847 (1997).
88. Nicotra, M.L. *et al.* A hypervariable invertebrate allodeterminant. *Curr. Biol.* **19**, 583–589 (2009).
89. Joly, E. Various hypotheses on MHC evolution suggested by the concerted evolution of CD94L and MHC class Ia molecules. *Biol. Direct* **1**, 3 (2006).
90. Zucchetti, I. *et al.* ciCD94–1, an ascidian multipurpose C-type lectin-like receptor expressed in Ciona intestinalis hemocytes and larval neural structures. *Differentiation* **76**, 267–282 (2008).
91. Flajnik, M.F. & Kasahara, M. Origin and evolution of the adaptive immune system: genetic events and selective pressures. *Nat. Rev. Genet* **11**, 47–59 (2010).
92. Guo, P. *et al.* Dual nature of the adaptive immune system in lampreys. *Nature* **459**, 796–801 (2009).

93. Uinuk-Ool, T.S. *et al.* Phylogeny of antigen-processing enzymes: cathepsins of a cephalochordate, an agnathan and a bony fish. *Scand. J. Immunol.* **58**, 436–448 (2003).
94. Uinuk-ool, T.S. *et al.* Identification and characterization of a TAP-family gene in the lamprey. *Immunogenetics* **55**, 38–48 (2003).
95. Tsutsui, S., Nakamura, O. & Watanabe, T. Lamprey (*Lethenteron japonicum*) IL-17 upregulated by LPS-stimulation in the skin cells. *Immunogenetics* **59**, 873–882 (2007).
96. von Andrian, U.H. & Mempel, T.R. Homing and cellular traffic in lymph nodes. *Nat. Rev. Immunol.* **3**, 867–878 (2003).
97. Galkina, E. *et al.* CXCR6 promotes atherosclerosis by supporting T-cell homing, interferon-γ production, and macrophage accumulation in the aortic wall. *Circulation* **116**, 1801–1811 (2007).
98. Latta, M., Mohan, K. & Issekutz, T.B. CXCR6 is expressed on T cells in both T helper type 1 (Th1) inflammation and allergen-induced Th2 lung inflammation but is only a weak mediator of chemotaxis. *Immunology* **121**, 555–564 (2007).
99. Diegelmann, J. *et al.* Expression and regulation of the chemokine CXCL16 in Crohn's disease and models of intestinal inflammation. *Inflamm. Bowel Dis.* **16**, 1871–1881 (2010).
100. Matsumura, S. *et al.* Radiation-induced CXCL16 release by breast cancer cells attracts effector T cells. *J. Immunol.* **181**, 3099–3107 (2008).
101. Liao, F. *et al.* STRL33, a novel chemokine receptor-like protein, functions as a fusion cofactor for both macrophage-tropic and T cell line-tropic HIV-1. *J. Exp. Med.* **185**, 2015–2023 (1997).
102. Heydtmann, M. *et al.* CXC chemokine ligand 16 promotes integrin-mediated adhesion of liver-infiltrating lymphocytes to cholangiocytes and hepatocytes within the inflamed human liver. *J. Immunol.* **174**, 1055–1062 (2005).
103. Sato, T. *et al.* Role for CXCR6 in recruitment of activated $CD8^+$ lymphocytes to inflamed liver. *J. Immunol.* **174**, 277–283 (2005).
104. Oh, S.T., Schramme, A., Tilgen, W., Gutwein, P. & Reichrath, J. Overexpression of CXCL16 in lesional psoriatic skin. *Dermatoendocrinol.* **1**, 114–118 (2009).
105. Martini, G. *et al.* CXCR6-CXCL16 interaction in the pathogenesis of Juvenile Idiopathic Arthritis. *Clin. Immunol.* **129**, 268–276 (2008).
106. van der Voort, R. *et al.* Elevated CXCL16 expression by synovial macrophages recruits memory T cells into rheumatoid joints. *Arthritis Rheum.* **52**, 1381–1391 (2005).
107. Nanki, T. *et al.* Pathogenic role of the CXCL16-CXCR6 pathway in rheumatoid arthritis. *Arthritis Rheum.* **52**, 3004–3014 (2005).