Human natural killer cells mediate adaptive immunity to viral antigens

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Adaptive immune responses are defined as antigen sensitization–dependent and antigen-specific responses leading to establishment of long-lived immunological memory. Although natural killer (NK) cells have traditionally been considered cells of the innate immune system, mounting evidence in mice and nonhuman primates warrants reconsideration of the existing paradigm that B and T cells are the sole mediators of adaptive immunity. However, it is currently unknown whether human NK cells can exhibit adaptive immune responses. We therefore tested whether human NK cells mediate adaptive immunity to virally encoded antigens using humanized mice and human volunteers. We found that human NK cells displayed vaccination-dependent, antigen-specific recall responses in vitro, when isolated from livers of humanized mice previously vaccinated with HIV-encoded envelope protein. Furthermore, we discovered that large numbers of cytotoxic NK cells with a tissue-resident phenotype were recruited to sites of varicella-zoster virus (VZV) skin test antigen challenge in VZV-experienced human volunteers. These NK-mediated recall responses in humans occurred decades after initial VZV exposure, demonstrating that NK memory in humans is long-lived. Our data demonstrate that human NK cells exhibit adaptive immune responses upon vaccination or infection. The existence of human memory NK cells may allow for the development of vaccination-based approaches capable of establishing potent NK-mediated memory functions contributing to host protection.

INTRODUCTION

Natural killer (NK) cells are an essential weapon of the immune system: They kill target cells rapidly and secrete large amounts of cytokines, and mutations preventing their maturation or function cause severe immune deficiencies (1). NK cells survey their environment with a diverse receptor repertoire including activating and inhibitory receptors, cytokine and chemokine receptors, and adhesion molecules (2). Healthy cells escape NK immune attack by expressing robust levels of major histocompatibility complex class I (MHC I) molecules that ligate NK-expressed inhibitory receptors, such as killer immunoglobulin-like receptors (KIRs), and cluster of differentiation (CD)94/NKG2A (3). However, infected or malignant cells often down-regulate MHC I while expressing ligands for NK-expressed activating receptors, such as NKG2D (4–6) and CD16 (7). Activation through these receptors elicits rapid target cell killing via the secretion of pore-forming proteins (perforin) and cytotoxic mediators (granzymes) that trigger target cell apoptosis (8, 9), as well as cytokine and chemokine release.

In humans, NK cells are identified as cells that express both the hematopoietic cell marker CD45 and the glycoprotein CD56 but not CD3, a signaling component of the T cell receptor (10, 11). Both T-box transcription factors T-bet and Eomesodermin (Eomes) are expressed by human NK cells, are crucial for NK development, are differentially expressed during specific developmental stages, and are in tissue-specific NK subsets (12, 13). Although NK cells were traditionally thought to develop in the bone marrow (BM), mounting evidence suggests that their progenitors can develop in situ. About 90% of human peripheral blood mononuclear cell (PBMC)–derived NK cells are T-bethi, Eomeslo, CD56lo, and CD16hi and are considered mature and highly cytotoxic. In contrast, the remaining 10% of human PBMC-derived NK cells are T-bethi, Eomeshi, CD56hi, and CD16lo (14) and exhibit robust cytokine production, but are considered less mature and less cytotoxic than CD56hi NK cells.

In contrast to PBMC, human tissues harbor an abundance of CD56hi, CD16lo NK cells (13, 15). Adult human spleen and liver contain both T-bet– and Eomes-expressing NK cells (13) and harbor NK cells that express the heterotrimeric GTP-binding protein–coupled C-X-C motif chemokine receptor 6 (CXCR6) (16), which is required for NK memory in mice (17–19). Eomes hi CXCR6 hi NK cells in human adult livers and spleens also express the activation or tissue residency marker CD69. Most of murine and human CXCR6–expressing NK cells are found in the liver, whereas CXCR6–expressing NK cells are largely absent from human PBMC (12, 15) and from murine spleen (17–19). Human spleen (17), BM, and lymph nodes (20), however, contain CXCR6+ NK cells but with perhaps a lower frequency than the human liver. In the steady state, the ligand for CXCR6, the transmembrane chemokine C-X-C motif ligand 16 (CXCL16), is abundantly expressed on liver sinusoidal endothelial cells in mice and humans, where it provides homing (16) and survival signals to CXCR6+ immune cells (18, 19, 21, 22). Whether human spleen, BM, and lymph nodes express significant amounts of human CXCL16 remains to be determined.

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Although NK cells have traditionally been considered cells of the innate immune system, mounting scientific evidence suggests that NK cells mediate adaptive immune responses (17–19, 23). Using delayed-type hypersensitivity (DTH) and viral challenge models (18, 19, 24), we previously reported that murine hepatic CXCR6hi NK cells exhibit all three hallmarks of adaptive immunity in response to haptons and virally encoded antigens: vaccination dependence, antigen specificity, and long-lived immunological memory (17–19). Further, our data and other published data over the past 12 years have demonstrated that NK memory in mice is B and T cell independent (18, 19, 24–26). Neither the priming of memory NK cells nor their memory response is dependent on T or B cells. Antigen-primed NK cells are sufficient and required for NK memory to occur as demonstrated by NK cell depletion or adoptive transfer (18, 19, 24, 25). The presence of naïve T and B cells neither augments nor inhibits memory NK cell–mediated recall responses, and the recruitment of antigen-primed NK cells to sites of challenge is antigen specific in mice, even in a competitive setting (18).

NK memory has been demonstrated in nonhuman primates (NHP) (23) and is, hence, not restricted to rodents. However, these exciting findings beg the question of whether human NK cells are capable of adaptive immune responses, which is currently unknown. We therefore tested whether human NK cells mediate adaptive immune responses using humanized mice and human volunteers. The BLT (BM, liver, and thymus) humanized mouse model we use for our studies is particularly suited to test our hypotheses because it allows for the production of several dozen human donor-matched BLT mice in which a functional human immune system develops, including human leukocyte antigen (HLA)–restricted T cells, as well as NK cells, B cells, dendritic cells (DC), neutrophils, and monocytes (27–32). Furthermore, BLT mice of a human donor-matched cohort are of identical murine and human genetics, allowing for direct comparisons of immune memory of donor-matched naïve versus vaccine-primed NK cells and controlled studies of immune responses to vaccination in tissues other than the peripheral blood.

Here, we demonstrate that spleens and livers of BLT mice harbor human NK cells that are tissue resident in phenotype and similar in phenotype to NK cells found in adult human liver and spleen. We demonstrate that human NK cells isolated from livers, but not from spleens, of vaccinated BLT mice mediate vaccination-dependent and antigen-specific killing in vitro. Evaluating varicella-zoster virus (VZV)–experienced human adults, we found that large numbers of NK cells are recruited to sites of VZV skin test antigen (VZV-STA) challenge, even decades after initial VZV exposure (33). These memory NK cells phenotypically resemble human tissue-resident NK. Our data demonstrate that human NK cells with a tissue-resident phenotype mediate adaptive immunity similarly to that of NK cells in mice and NHP.

RESULTS

Spleens and livers of BLT mice harbor tissue-specific human NK cells

Human NK cells develop in BLT mice; they are responsive to interleukin-15 (IL-15), a cytokine important for NK cell development and function (34); kill MHC I–deficient target cells; and produce interferon-γ (IFN-γ) upon activation (35). However, little is currently known about the distribution of tissue-specific NK cells in BLT mice and their maturation status. We therefore used multiparametric flow cytometry to examine the expression of tissue-specific transcription factors and cell surface markers on splenic and hepatic BLT-derived NK cells 4 to 6 months after reconstitution. Eight to 12 weeks after reconstitution, more than 90% of hematopoietic cells found in PBMC of our BLT mice were of human origin (fig. S1A), and human NK cells were present systemically, including in BLT spleens and livers (fig. S1, B and C). Four to six months after reconstitution, both T-bet– and Eomes-expressing NK cells were present in spleens and livers of BLT mice (Fig. 1A). Eomes hi CD56 hi CXCR6 hi NK cells were more abundant in BLT mouse liver, whereas BLT-derived splenic NK cells were more frequently T-bet hi CD56lo and CXCR6lo, similar to reports examining human adult splenic or hepatic NK cells (13, 15). We next inspected the expression of tissue residency and functional markers on splenic and hepatic BLT-derived NK cells (Fig. 1B). Spleen NK cells had a lower frequency of CD56lo NK cells than liver NK cells, as well as a reduced frequency of CXCR6, CD16, NKG2D, and CX3CR1-expressing NK cells, albeit all of these markers were nevertheless expressed on a substantial number of splenic NK cells (Fig. 1B). However, we observed no differences in the frequencies of CD69, CD62L, perforin, granzyme B–, or IFN-γ–expressing NK cells between BLT spleens and livers (Fig. 1B). We conclude that expression of tissue residency markers, lymph node–homing ability, cytotoxic effector functions, and IFN-γ production is similar between splenic and hepatic NK cells, whereas the expression of the activating receptors NKG2D and CD16 is reduced on splenic BLT-derived NK cells compared with donor-matched hepatic NK cells.

Splenic and hepatic NK cells are similar in phenotype in BLT mice and human adults

We next compared the phenotypes of BLT–derived splenic and hepatic NK cells with that of human adult donor-derived spleen or liver and with the current literature (13, 15). Liver samples were of transplant quality, whereas spleen samples were obtained from newly diagnosed and treatment-naïve patients with pancreatic cancer undergoing splenectomy. Only disease-free spleens were used for experimental analyses. With the exception of CD62L, the frequency of CD56hi, CXCR6, CD16, NKG2D, CD94, CD69, NKG2A, CX3CR1, perforin, and granzyme B–positive NK cells was indistinguishable between BLT–derived and adult human donor-derived livers (Fig. 2A). Likewise, splenic BLT–derived NK cells were similar to human spleen–derived NK cells, with the exception of the frequencies of CD16, NKG2D, and CD62L-expressing NK cells, which were reduced in BLT mice compared with human adult spleen (Fig. 2B). However, these markers were nevertheless robustly expressed on a substantial number of splenic BLT–derived NK cells. Because BLT mice do not develop proper lymph node architecture, it may not be unexpected that the frequency of CD62L–expressing NK cells is higher in BLT mice compared with human adult liver and spleen, as these cells are left to circulate in BLT mice but may home to lymph nodes in healthy adult humans. We conclude that human NK cells in BLT mice phenotypically resemble the corresponding tissue-specific NK cells of adult humans, both in the expression of tissue-specific transcription factors and relevant cell surface markers.

Phenotypically mature human NK cells develop in BLT mice

We next used cytomtery by time–of–flight (CyTOF) mass cytometry to compare the expression of additional markers on BLT NK cells
versus human adult PBMC NK cells, a population we consider predominantly mature in phenotype (fig. S2 and table S1). We found that the inhibitory and activating KIR2DL1, KIR2DL3, KIR2DL5, KIR2DS2, KIRDS4, and KIR3DL1 receptors; the activating and costimulatory receptors NKp30, NKp40, NKp46, DNAX accessory molecule-1 (DNAM-1), 2B4, CD8, NK-T-B-antigen (NTB-A), and Tactile; the inhibitory receptors CD94/NKG2A and PD-1; and the apoptosis-inducing FasL were expressed at similar frequencies on BLT-derived versus human adult PBMC-derived NK cells (fig. S2). In contrast, the frequencies of CD94/NKG2C-, CD57-, and T-cell immunoreceptor with Ig and ITIM domains (TIGIT)--expressing human NK cells were reduced in BLT mice compared with adult human PBMC-derived NK cells. Because CD57 is a marker for terminally differentiated NKG2C+ NK cells that accumulate in cytomegalovirus (CMV)--seropositive human adults (36), and TIGIT is a marker of immune exhaustion (37, 38), we did not expect either marker to be robustly expressed in naïve BLT mice. Thus, we conclude that BLT-derived human splenic and hepatic NK cells are similar in phenotype to human adult tissue-matched donor NK cells, supporting the use of this model to analyze whether human NK cells mediate priming-dependent and antigen-specific immunological memory responses after vaccination.

**Human hepatic and splenic NK cells respond to vaccination of BLT mice**

Whether human NK cells are capable of adaptive immunity has not yet been determined. We chose humanized mice for the testing of two of the three hallmarks of adaptive immunity: vaccination dependence and antigen specificity (17, 19), as the experimental use of donor-matched cohorts of naïve and vaccinated humanized mice...
allows us to compare antigen-specific recall responses of truly antigen-naive NK cells with that of donor-matched, antigen-primed NK cells. Such comparisons would be difficult to perform with human samples because it is not possible to trace the previous antigen exposure of adult humans. To test whether human NK cells in BLT mice respond to vaccination, we vaccinated BLT mice by intravenous and intra-peritoneal infusions of syngeneic DC preparations that were loaded for 2 hours in a tissue culture incubator with HIV envelope protein (HIV-Env; from HIV-Q23-17 clone) and left donor-matched control BLT mice naïve by infusing them with the same preparation of donormatched DC but without HIV-Env loading. This protocol elicits strong immunological memory responses in mice (18). We chose HIV-Env, because both murine and NHP-derived NK cells mediate adaptive immune responses to this clinically important antigen (18, 23), and used a mix of monomeric gp120 and trimeric gp140 for these studies, because it is currently unclear which antigen confirmation triggers superior immunological memory in human NK cells. Fourteen days after vaccination, we examined the phenotypes of splenic and hepatic

![Fig. 2. Spleens and livers of BLT mice harbor human NK cells similar in phenotype to human adult tissue-matched NK cells.](image-url)
NK cells isolated from either control or vaccinated human donor-matched BLT mice using CyTOF (fig. S3). HIV-Env–primed splenic and hepatic NK cells had decreased expression of select activating receptors and maturation markers (CD16 and CD57) and inhibitory receptors (KIR2DL1 and KIR2DL5) and had increased expression of the adhesion molecules CD2 and CD62L. Both splenic and hepatic NK cells further up-regulated the inhibitory receptor NKG2A, but only splenic NK cells up-regulated perforin. Splenic NK cells, unlike hepatic NK cells, also decreased their expression of the adenosine-diphosphate-ribosyl cyclase (39), the adhesion molecule CD96, and the activating KIRs KIR2DS4 and KIR2DS5 (fig. S3). We conclude that splenic and hepatic NK cells in BLT mice respond to DC-based HIV-Env vaccination by global and tissue-specific changes in their expression levels of activating and inhibitory receptors, adhesion molecules, and effector function molecules.

**Human NK cells mediate antigen-specific and vaccination-dependent recall responses**

To test whether splenic or hepatic NK cells mediate vaccination-dependent and antigen-specific recall responses upon vaccination of the host, we performed in vitro killing assays with splenic or hepatic NK cells from human donor-matched naïve or HIV-Env–vaccinated BLT mice. All groups of BLT mice were treated weekly with trans-presented human IL-15 before immunization to significantly expand the numbers of NK cells in both the spleen and liver of BLT mice, thereby enabling replicate-well experiments (fig. S4). BLT mice were left naïve, immunized via intraperitoneal injection with antigen-free syngeneic DC as a control, or immunized with recombinant HIV-Q23-17 Env (gp140/gp120)–loaded syngeneic DC. Fourteen days after immunization, human NK cells were isolated from the spleens and livers of naïve and HIV-Env–vaccinated human donor-matched BLT mice by flow cytometry–based cell sorting, and the amount of antigen-specific NK-mediated killing was determined by comparing killing of syngeneic target cells (DC) by naïve versus HIV-Env–primed NK cells. Target cells were either loaded with the same HIV-Env preparation used for vaccination (experimental “recall” group) or used as control target cells that were (i) left antigen free (vaccination requirement control); (ii) loaded with an irrelevant protein antigen, ovalbumin (Ova; antigen specificity control no. 1); or (iii) loaded with an irrelevant pathogen, ultraviolet (UV)–inactivated influenza A virus H1N1 PR8 (UV-inactivated H1N1 PR8; antigen specificity control no. 2). Human BLT liver- or spleen-derived NK cells were cocultured with carboxyfluorescein diacetate succinimidyl ester (CFSE)–labeled syngeneic target cells at a 1:1 ratio, for 6 hours at 37°C 5% CO₂ before target cell killing was determined using flow cytometry. We found that HIV-Env–primed hepatic NK cells vigorously killed HIV-Env–loaded syngeneic target cells (DC) in vitro (Fig. 3A). The killing of HIV-Env–loaded syngeneic target cells by hepatic NK cells was antigen specific because hepatic NK cells from HIV-Env–vaccinated animals did not kill syngeneic target cells loaded with either UV-inactivated H1N1 PR8 or Ova nor did they kill antigen-free targets. Killing assays were free of T cells as demonstrated by post-sort analysis (fig. S5). Splenic NK cells, isolated from HIV-Env–vaccinated donors, did not kill HIV-Env–loaded syngeneic targets, despite their similar expression of perforin and granzyme B, when compared with hepatic HIV-Env–specific memory NK cells (Fig. 1B), and, as expected, neither did naïve hepatic nor did naïve splenic NK cells (Fig. 3). We did not observe killing of antigen-free (“none”) target cells by splenic or hepatic NK cells because their death rate was similar to that of target cells incubated without NK cells for the duration of the assay, and hence, we are not subtracting significant background killing from our antigen-loaded experimental killing assay group’s results. Our findings are similar to previously published results in mice, in which NK memory is also restricted to hepatic NK cells (18). We conclude that, in BLT mice, hepatic human NK cells mediate vaccination-dependent, antigen-specific recall responses, both hallmarks of adaptive immunity.

We next analyzed the expression of CXCR6 on splenic and hepatic human NK cells of BLT mice because the survival and memory functions of murine hepatic NK cells are dependent on NK cell–expressed CXCR6 (18). We found that most of hepatic but not splenic NK cells express CXCR6 (Figs. 1 and 2). Further, BLT-derived hepatic NK cells had similar CXCR6 expression levels and frequencies to human NK cells isolated from healthy human liver (15). Thus, in mice and humanized mice, a large percentage of hepatic NK cells expressed CXCR6, which localizes these cells to the liver, and hepatic NK cells from mice and humanized mice mediate vaccination-dependent and antigen-specific immunological memory to HIV-Env.

**Human NK memory is very long-lived**

To test whether human NK cells are capable of the third hallmark of adaptive immunity and longevity, we elicited a DTH response, a hallmark of adaptive immunity, in the skin of VZV-experienced human volunteers via intradermal injection of VZV-STA. To maximize time elapsed between NK priming by VZV infection and VZV-STA challenge, we excluded volunteers who had received Zostavax (Merck) vaccinations or had a history of shingles. This DTH skin test allows us to examine whether human NK cells are recalled to sites of VZV-STA challenge, decades after the presumed time of NK cell priming to VZV antigen, and to determine memory NK cell phenotypes and effector functions. We chose VZV for our study because childhood infection with VZV generally confers lifelong immunity in healthy persons, and NK cells respond to VZV infection (40, 41) and have been implicated in host protection from VZV disease (1, 41, 42).

Healthy human volunteers, who were between 22 and 65 years of age and had previously suffered from VZV infection in their youth, were injected intradermally with VZV-STA. As a control, sterile saline solution was injected in the opposite arm, and VZV-STA and control blister fluids were analyzed at the same time. We scored the magnitude of the DTH response at all injection sites 3 days after VZV-STA or saline challenge by measuring the erythema, induration, and diameter at the site of injection to produce a clinical score as previously described (33, 43–45). All VZV-STA–challenged donors scored between 3 and 7 on a scale of 1 to 10 (maximum score, 10), with higher responses noted in younger individuals as previously reported (44). To avoid contamination of our experimental samples with blood-derived NK cells, we did not perform a punch biopsy on the DTH site but instead generated a skin blister above the site of VZV antigen challenge because blister fluid is mostly devoid of PBMC (33, 44, 45). Sixteen hours later, we compared NK cell frequencies and phenotypes in the blister fluids from naïve control and VZV-STA–injected skin with each other and with donor-matched PBMC–derived NK. Although we did not detect NK cells in blister fluids from the sites of intradermal saline control injections, large numbers of NK cells infiltrated blister fluids above the sites of VZV-STA in all donors, including donors that experienced VZV infection many decades earlier (Fig. 4, A and B).
We next analyzed the cytotoxic functions of VZV blister fluid–derived human NK cells by assessing their CD107a expression levels (46–48) ex vivo, as CD107a is a functional marker for NK cytotoxicity (23). Our approach is supported by multiple reports demonstrating that cytokine release does not up-regulate CD107a expression on the surface of NK cells but that up-regulation correlates significantly with NK-mediated cytotoxicity (47, 48). Further, a statistically significant correlation between NK killing of HIV-Gag– or HIV-Env–primed target cells and CD107a up-regulation has been reported for memory NHP-derived NK cells, supporting our conclusion that CD107a up-regulation on NK cells is indicative of antigen-specific cytotoxicity (23). To take our “degranulation snapshot,” we compared the frequency of CD107a-expressing NK cells in freshly harvested VZV-STA challenge blister fluids with that of donor-matched PBMC-derived NK cells because naïve skin-associated blister fluid did not contain sufficient numbers of NK cells for proper statistical analysis (Fig. 4A). We found that NK cells isolated from VZV-STA challenge blister fluid expressed significant levels of cell surface CD107a directly ex vivo, without NK restimulation or blockade of CD107a internalization, when compared with human donor-matched PBMC NK cells (Fig. 4C). These data are important because they demonstrate that NK cells isolated from the site of an antigen recall response are actively killing target cells in blister fluid of VZV-STA challenge. Because NK cells at sites of VZV-STA challenge are cytotoxic, they are unlikely to be bystander immune cells recruited by elevated cytokines or chemokines at the challenge site in skin, as cytokine and chemokine signaling alone does not trigger NK-mediated killing (49). We conclude that NK cells recruited to the site of VZV-STA challenge in VZV immune donors are cytotoxic and distinct both in phenotype and function from donor-matched PBMC-derived NK cells. Their rapid recruitment to sites of VZV-STA challenge 30 years after initial infection of the host suggests that NK cells play a role in long-term antigen-specific recall responses in humans.

Fig. 3. Human hepatic NK cells mediate antigen-specific and vaccination-dependent killing. Human donor-matched BLT mice were left naïve or were immunized by intraperitoneal and intravenous injections with recombinant HIV-Q23-17 Env (gp140/gp120)–loaded syngeneic DCs (HIV-Env). Fourteen days after the immunization, human NK cells were isolated from either naïve or HIV-Env–vaccinated human donor-matched BLT mice by flow cytometry–based cell sorting. The NK cells were cocultured with CFSE-labeled, antigen-free, Ova-loaded, UV-inactivated H1N1 PR8 influenza A– or HIV-Env–loaded syngeneic target cells at a 1:1 ratio for 6 hours at 37°C, 5% CO2, before target cell killing was determined using flow cytometry. A total of three (spleen) to four (liver) genetically unrelated human donor cohorts of five to eight BLT mice were analyzed 5 months after transplantation, and the data were pooled for (A) and (B). Two-way ANOVA with Tukey’s multiple comparisons test; ****P < 0.0001.
Human memory NK cells express tissue residency markers

We next compared the expression of tissue-resident NK markers and the expression of the transcription factors T-bet and Eomes on cytotoxic (CD107a⁺) versus noncytotoxic (CD107a⁻) NK cells in VZV-STA–challenged blister fluids (Fig. 5, A and B). We found that CD107a⁺ VZV-STA blister fluid NK cells were CD56hi and expressed more CXCR6, NKG2D, and CD69 than CD107a⁻ NK cells, whereas CD62L expression was reduced on CD107a⁺ VZV-STA blister fluid NK cells (Fig. 5, A and B), and CD16 expression was similar between both types of blister fluid NK cells. This may not be unexpected because CD16 (FcyRIII) mediates cytotoxicity in an antibody-dependent way, and NK memory is independent of B cells (17–19). VZV-STA blister fluid NK cells, when analyzed in bulk and compared with donor-matched PBMC-derived NK cells, were similarly distinct in their tissue-resident phenotype. NK cells at sites of VZV-STA challenge were predominantly CD56hi and more frequently expressed CXCR6, NKG2D, CD69, and CD62L, whereas the expression of the cytotoxicity receptor CD16 was significantly reduced on VZV-STA–challenged blister fluid NK cells compared with donor-matched PBMC NK cells (Fig. 5C). VZV skin antigen challenge blister fluid NK cells expressed the transcription factor Eomes, with or without coexpression of T-bet, whereas donor-matched PBMC-derived NK cells were Eomes⁺ and generally expressed T-bet either with or without Eomes coexpression (Fig. 5D). Although numerous publications have investigated the phenotypes and effector functions of human NK cells in inflamed skin, the phenotype of healthy skin-resident NK cells remains elusive, in large part due to a natural lack of NK cells in uninfamed skin. A single publication describes skin-resident NK cells as NKG2D- and perforin-negative and were unable to lyse target cells unless preactivated with IL-2 (50). In contrast, VZV-STA blister fluid NK cells express significant amounts of NKG2D and degranulate ex vivo (Fig. 5, A to C). We conclude from our data that human NK cells recruited to the site of VZV-STA challenge phenotypically resemble activated human tissue-resident NK cells and participate in lifelong immune memory responses.

DISCUSSION

Currently, vaccinations are the best way to protect public health, having saved more than 10 million lives globally in the past 55 years (51). Although there is still no preventative HIV vaccine, mounting epidemiological and experimental evidence points to an important role for NK cell–mediated effector functions in host protection from HIV infection (52, 53). Should human NK cells mediate adaptive immune responses more broadly to pathogen-encoded or tumor antigens, then the discovery of NK memory opens the door for vaccination to direct potent memory NK cell–mediated effector functions toward host protection, including protection from HIV, which will require a rapid host protective immune response. We therefore evaluated whether human NK cells mediate three hallmarks of adaptive immunity to HIV-Env: antigen sensitization dependence, antigen specificity, and longevity. Using BLT mice and human volunteers, we discovered that human NK cells mediate robust vaccination-dependent recall responses featuring antigen specificity and longevity.

We performed deep phenotyping of splenic and hepatic BLT-derived NK cells using CyTOF and multiparametric flow cytometry. We discovered that the expression of maturation markers, activating receptors, and inhibitory receptors was mostly consistent between NK cells from spleens and livers of BLT mice and adult human PBMC-derived NK cells. Similarly, the expression of NK cell–associated transcription factors and tissue-specific markers for splenic and hepatic NK cells was indistinguishable between human and BLT-derived NK cells, when compared with adult human spleen- and liver-derived NK cells (Fig. 2). Our data are important in the context of a previous report that describes BLT mouse BM-derived human NK cells as phenotypically immature, 8 weeks after reconstitution of NOD/scid/IL2Rγ-deficient (NSG) mice (34). In contrast, we phenotyped splenic and hepatic NK cells, rather than BM-derived human NK cells, and did so 4 to 6 months after transplantation. Thus, either human NK cells are more mature in spleens and livers of BLT mice and adult human PBMC-derived NK cells. Similarly, the expression of NK cell–associated transcription factors and tissue-specific markers for splenic and hepatic NK cells was indistinguishable between human and BLT-derived NK cells, when compared with adult human spleen- and liver-derived NK cells (Fig. 2). Our data are important in the context of a previous report that describes BLT mouse BM-derived human NK cells as phenotypically immature, 8 weeks after reconstitution of NOD/scid/IL2Rγ-deficient (NSG) mice (34). In contrast, we phenotyped splenic and hepatic NK cells, rather than BM-derived human NK cells, and did so 4 to 6 months after transplantation. Thus, either human NK cells are more mature in spleens and livers of BLT mice than in the BM or they simply mature over time. In the previously mentioned report, the maturation of human NK cells was only achieved upon infusions of BLT mice with trans-presented human IL-15 (34), whereas in our study, infusions with trans-presented IL-15 were not required to mature NK cells but to increase their numbers (fig. S4). Because BLT mice bear xenografts of human lymphoid tissue and have limited blood volume, we did not perform skin-based DTH experiments nor did we extensively phenotype BLT-derived PBMC. Despite these caveats, our data support the conclusion that BLT mice harbor mature,
functional human NK cells that not only resemble human NK cells in phenotype but also can be primed to become HIV-Env–specific memory NK cells that mediate vaccination-dependent antigen-specific recall responses. When we challenged the skin of VZV-immune volunteers with VZV-STA and examined NK cells recruited to sites of VZV-DTH, we found large numbers of NK cells at sites of VZV-DTH but minimal NK cell recruitment to saline control challenge (Fig. 4, A and B). NK cells recruited to sites of VZV-DTH were cytotoxic (Fig. 4C). We hypothesize that cytotoxic NK cells at sites of VZV skin antigen challenge are killing VZV-loaded target cells of unknown identity because cytokine release does not up-regulate CD107a on NK cells and elevated cytokine or chemokine levels that likely exist at sites of VZV-DTH cannot, by themselves, trigger NK-mediated cytotoxicity, which requires NK cell activation via the ligation of NK activating receptors (49). The precise nature of these target cells is currently unknown; however, it is tempting to speculate that they may be antigen-presenting cells loaded with injected VZV glycoproteins. Unfortunately, because our study protocol did not include approval to inject volunteers with irrelevant control antigens or to perform VZV-STA challenge in VZV-naïve persons, who are quite rare, our hypothesis cannot be experimentally addressed further at this time. Similar to our previous reports for murine memory NK cells, human memory NK cells resembled tissue-resident NK cells based on their phenotypes and expressed transcription factors (13, 15) and were distinct in phenotype when compared with human donor-matched PBMC-derived NK cells (Fig. 5). However, because both human spleen- and liver-derived NK cells include CXCR6 + CD16lo CD69 + NKG2D + CD62L + T-betlo and Eomeshi NK cells (13, 15) and CXCR6 expression is not restricted to these tissues in humans (20), it is currently impossible to distinguish whether human memory NK cells originate from the liver or another organ. We were eager to determine whether CXCR6 expression is required for the function and survival of human memory NK. Unfortunately, we found that modulation of the CXCR6-CXCL16 pathway via antibody binding compromised NK viability, thereby preventing us from performing this experiment.

Our findings obtained in BLT mice are only partially in agreement to those reported for NHP, where both hepatic and splenic NK cells mediated antigen-specific recall responses (23). However, a direct phenotypic comparison of macaque, human, BLT mouse, and

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**Fig. 5. Human NK memory is mediated by NK cells with a hepatic phenotype.** Human donor-matched PBMC and blister fluids were isolated on day 3 after VZV glycoprotein challenge and stained for indicated markers. (A and B) Analysis of NK-expressed markers on degranulating (CD107a +) versus nondegranulating (CD107a−) NK cells in blister fluid of VZV-STA–injected skin. An example of a histogram overlay for each marker is shown in (A) for a single donor, whereas five to seven genetically unrelated human donors were individually analyzed for each marker in (B). (C) Frequency of human NK cells expressing the indicated markers indicative of a human hepatic NK cell phenotype. Six to 11 genetically unrelated human donors were individually analyzed for each marker. (D) Frequency of human NK cells expressing the indicated transcription factor master regulators indicative of a human hepatic NK cell phenotype. Six genetically unrelated human donors were individually analyzed for each marker. Paired t test: *P = 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001.
murine NK cells remains elusive due to significant species-specific differences in the expression of NK-specific cell surface markers, differences in the availability of antibodies specific to these markers for each species, and, hence, differences in the way NK cells are identified and isolated for experimental analysis. Reports that analyze the phenotypes of human splenic or hepatic NK cells are rare (13, 15); thus, it is currently unclear how related human splenic and hepatic NK cells may be in humans versus BLT mice, where the frequency of NKG2D- and CD16 expressing NK cells was reduced, whereas the frequency of perforin- and granzyme B–expressing NK cells was similar (Fig. 2B). This leaves the possibilities that memory NK cells in mice and BLT mice are more restricted in their tissue residency, perhaps through a species-specific difference in the expression of ligands important for memory NK cell survival or function, such as CXCL16, whereas human splenic and hepatic NK cells are similar, thus indistinguishable from each other in VZV-STA blisters. Alternatively, NK cells in humans and NHP could be distinct, with human NK memory restricted to hepatic NK cells only in humans, BLT mice, and mice. The answer to these important questions will require further studies.

Whether NK memory responses in primates are modulated by other immune cell types is currently unknown, and this important question cannot be easily experimentally assessed in humans. In mice, neither antigen sensitization nor NK cell–mediated recall responses require the presence of T or B cells, nor are they inhibited by the presence of naïve T or B cells (17–19, 26). However, we cannot formally exclude the possibility that, in humans, other immune cells may modulate NK cells and their memory responses through unknown mechanisms because this is not experimentally testable in vivo. It is, however, important to note that IFN-γ release by NK cells is not a measure of NK memory because naïve NK cells and even umbilical cord blood–derived NK cells are robust IFN-γ producers (54). The mechanisms by which NK cells recognize and distinguish different antigens remain unknown, were not the focus of this study, and were likely distinct from that of NKG2C-expressing NK cells that expand upon CMV infection of humans (36, 55). However, this germline-encoded one gene—one agonist activation and expansion may be distinct in its mechanism from that of the human memory NK cells described here, which we hypothesize is the result of fixed genomic rearrangements, rather than epigenetic modifications (17). Future work will need to be directed toward understanding how long-lived, antigen-specific NK memory responses can be targeted toward improved human health via the development of novel clinical diagnostic approaches, vaccines, therapeutic agents, or immunotherapies.

### MATERIALS AND METHODS

#### Study design

The goals of this study were (i) to investigate the maturation and functional status of human NK cells in the spleens and livers of naïve BLT mice, via direct comparisons of NK cell–expressed cell surface markers and transcription factors between BLT-derived NK cells of the spleen and liver to that of adult human spleen- and liver-derived NK cells, as well as human adult–derived PBMC NK cells; (ii) to investigate whether human splenic or hepatic NK cells mediate vaccination-dependent and antigen-specific recall responses to experimental antiviral vaccines using BLT mice; and (iii) to investigate the longevity of the adaptive NK cell–mediated immune response in human volunteers via DTH and examination of responder NK cells at sites of challenge. A minimum of three genetically unrelated human donor-derived BLT cohorts with a minimum of five mice per group were analyzed for each data point, with the exception of figs. S2 and S3, for which five mice were pooled to obtain sufficient numbers of NK for CyTOF analysis. We used three to seven genetically unrelated human donors with four to nine mice per group to analyze NK cell phenotypes and NK cell–mediated effector functions by flow cytometry and functional assays. We further used 11 genetically unrelated human volunteers to analyze the longevity of the human NK cell–mediated immunological recall response to VZV skin antigen challenge using skin blister fluid–derived NK cells for our experimental evaluations, as well as human donor-matched PBMC-derived NK cells as a control.

#### Animals

BLT mice were generated as previously described (29). Briefly, 6- to 12-week-old female lymphocyte-deficient NSG mice were transplanted underneath the capsule of the left kidney with small pieces of human fetal liver and thymus (Advanced Bioscience Resources). Immediately after transplantation, NSG recipient mice were infused with 1.5 × 10^5 to 4 × 10^5 autologous fetal liver–derived CD34^+ hematopoietic stem cells (HSCs) via the mouse tail vein. Twelve weeks after transplantation and HSC infusion, the reconstitution efficiency of each animal was determined by flow cytometric analysis of PBMC collected from the submandibular vein. PBMCs were isolated by density gradient centrifugation using Ficoll-Paque (GE Healthcare) as per the manufacturer’s protocol, washed once in sterile phosphate-buffered saline (PBS), supplemented with 2% fetal bovine serum (FBS), resuspended in 0.2 ml of PBS supplemented with 2% FBS, incubated with murine and human Fc Block [Becton Dickinson (BD)] for 10 min at 4°C to prevent nonspecific antibody staining, stained with antibodies specific to murine CD45 (30-F11; BioLegend, GE Healthcare) and human CD45 (H130; BioLegend), and evaluated for their percentage of human CD45^+ by flow cytometry. Mice with ≥60% reconstitution with human CD45^+ hematopoietic cells were used for experiments. All mice were cared for in the animal facilities of the Center for Comparative Medicine at Baylor College of Medicine (BCM) and Texas Children’s Hospital, and all protocols were approved by the BCM Institutional Animal Care and Use Committee.

#### Isolation of human CD34^+ HSC from fetal liver

A single-cell suspension was generated from human fetal liver by mechanical disruption using a 40-μm nylon mesh. Cells were washed in sterile PBS supplemented with 2% FBS, and CD34^+ cells were isolated via a CD34-positive selection kit (STEMCELL Technologies) as per the manufacturer’s protocol.

#### Generation of HIV-Env glycoproteins

The sequence for the envelope gene (env) from HIV-Q23-17, a clade A Kenyan isolate of HIV-1, was motif-optimized and synthesized as the gene encoding gp140 with the cleavage site intact between gp120 and gp41, truncated just before the transmembrane domain of gp41. This env gene was cloned into the mammalian cell expression vector pEMC*, modified for enhanced expression under the control of the human CMV immediate−1 promoter and enhancer (56). Protein expression was performed by transient transfection of human 293 cells, generating a mixture of soluble, secreted Env gp140 and Env...
gp120 upon partial cleavage of gp140. The protein was purified using lectin affinity chromatography, followed by sizing chromatography to yield protein that was 95% pure (Coomassie gel staining) (57); identity was confirmed by Western blot using HIV-Env–specific human monoclonal antibody b12.

**IL-15 treatment of BLT mice used as donors for NK cell killing assays**

Before vaccination, BLT mice were treated weekly, for 4 weeks, with human IL-15RaFc chimera (2.5 μg per mouse; R&D) and recombinant human IL-15 (2.5 μg per mouse; BioLegend) to expand their mature NK populations. IL-15 injections were given intraperitoneally using a 30-gauge needle.

**Vaccination of BLT mice**

Spleens from two to three donor-matched naïve BLT mice were processed into single-cell suspensions by mechanical disruption. Immune cells were isolated by density gradient centrifugation using Ficoll-Paque (GE Healthcare) as per the manufacturer’s protocol (950g, 20 min, and no brake) and washed once in sterile PBS supplemented with 2% FBS. Splenocytes (5 × 10^5 to 7.5 × 10^5) were plated in 200 μl of RPMI 1640, supplemented with 10% FBS, and 1% of each of the following: Hepes, nonessential amino acids, Na-pyruvate, l-glutamine, penicillin-streptomycin, (R-10 medium) in a V-bottom plate. To each well, 20 μg of HIV-Env (Q23-17 HIV-1 encoded monomeric gp120 + trimeric gp140), Ova, or UV-inactivated influenza A virus PR8 H1N1 was added, and cells were incubated for 2 hours at 37°C, 5% CO_2_. Cells were then divided into equal amounts and injected intravenously and intraperitoneally into donor-matched BLT mice using a 30-gauge needle.

**In vitro killing assay**

Target cell isolation from spleens of naïve BLT mice for

Spleens were isolated from euthanized naïve BLT donor mice and mechanically disrupted in sterile PBS 2% FBS using frosted glass slides to generate a single-cell suspension. Immune cells were isolated by density gradient centrifugation using Ficoll-Paque (GE Healthcare) as per the manufacturer’s protocol (950g, 20 min, and no brake). Immune cells were washed once in sterile PBS supplemented with 2% FBS, resuspended in 150 μl of PBS 2% FBS, incubated with murine and human Fc Block (BD) for 10 min at 4°C, and stained with antibodies specific to murine CD45 (30-F11; BioLegend) and human CD43 (CD43-10G7; BioLegend). CD43^+ target cells (B cells and DCs) were isolated using fluorescence-activated cell sorting (FACS) on a BD Aria, resulting in ≥99.8% purity.

**Isolation of human NK cells from BLT mouse livers and spleens**

Fourteen days after immunization, single-cell suspensions from spleens and livers were generated by mechanical disruption of spleens, using frosted glass slides, and livers, using a 40-μm nylon mesh. Immune cells were then isolated by density gradient centrifugation using Ficoll-Paque (GE Healthcare) as per the manufacturer’s protocol. Splenic and hepatic immune cells were washed once in sterile PBS 2% FBS, resuspended in 150 μl of PBS 2% FBS, incubated with murine and human Fc Block (BD) for 10 min at 4°C, and stained with antibodies specific to mCD45 (30-F11; BioLegend), hCD45 (H130; BioLegend), hCD3 (OKT3; BioLegend), and hCD56 (HCD56; BioLegend) for 30 min at 4°C. Human NK cells (≥99% pure) were isolated by FACS-based cell sorting of mCD45/hCD3^+ hCD45/ hCD56^+ NK cells using a BD Aria (5000 to 7000 events/s).

**Research ethics approval for PBMC, adult liver, and spleen tissue**

All human peripheral blood and adult spleen samples were obtained by written informed consent, and the protocol was approved for use by the National Institutes of Health and BCM Institutional Review Boards for the Protection of Human Subjects and in accordance with the Declaration of Helsinki.

**PBMC from healthy donors**

All PBMC donors were healthy adult volunteers. To isolate PBMC, peripheral blood was diluted 1:1 with Ca^{++}- and Mg^{++}-free PBS and layered over Ficoll-Paque (GE Healthcare), centrifuged at 2000 rpm with no brake for 20 min at room temperature according to the manufacturer’s instructions. The lymphocyte layer was removed, washed, and counted, and immune cells were stained with the indicated antibodies and analyzed using multiparametric flow cytometry. No frozen cells were used for this study.

**Adult liver and spleen tissue**

Informed patient consent was not required to collect human adult liver perfusates from adult cadaver donors. Liver perfusate (50 to 100 ml) was collected and centrifuged, and the cell pellet was resuspended in RPMI 1640 (HyClone) and separated by density centrifugation using Ficoll-Paque (GE Healthcare) as described above. Cells were washed in PBS, counted, and used immediately for multi-parameter flow cytometry. Adult spleen tissue was obtained from patients undergoing pancreatic ductal adenocarcinoma surgery or splenectomy related to chronic pancreatitis. Patients had no known viral infections, were treatment naïve, and did not have involvement of the spleen with cancer. Single-cell suspensions were generated from adult spleen tissue by mechanical separation, centrifugation, and density centrifugation over Ficoll-Paque (GE Healthcare). The lymphocyte layer was removed, washed, and counted, and immune cells were stained with the indicated antibodies and analyzed using multiparametric flow cytometry. No frozen cells were used for this study.

**In vitro NK cell killing assay**

Target cells were plated in 96-well V-bottom plates at 5 × 10^5 cells per well in R-10 media, either pulsed with Ova (5 μg per well), UV-inactivated influenza A H1N1 PR8 (5 μg per well), or HIV-Env (5 μg per well) or left antigen-free, and incubated for 24 hours at 37°C, 5% CO_2_. Cells were then washed twice with sterile PBS and labeled with CFSE (CellTrace, Thermo Fisher Scientific) as per the manufacturer’s protocol for subsequent identification by flow cytometry and washed twice with R-10 media. CFSE-labeled target cells were cocultured in V-bottom plates with sorted hepatic or splenic syngeneic human NK cells at a 1:1 ratio for 6 hours at 37°C 5% CO_2. After the 6-hour incubation period, plates were immediately placed on ice. NK/target cell ratios were determined by flow cytometric analyses using pure populations of CFSE-labeled target cells or CD56-stained NK cells as fluorescence minus one (FMO) controls. NK cell killing was calculated as follows for either naïve or HIV-Env–primed NK cells: percent specific lysis = [1 – (antigen–free target cell ratio/antigen-loaded target cell ratio)] × 100, whereby antigen loading was performed with Ova protein, UV-inactivated influenza A H1N1 PR8, or HIV-Env.
Staining and mass cytometry acquisition
The staining antibody panel is outlined in table S1. All antibodies were conjugated using Maxpar X8 labeling kits (Fluidigm). Antibodies were lyophilized in single-use LypoSpheres (BIOLYPH, Chaska, MN) to ensure antibody stability. Example of stains and gating strategy for each marker is shown in fig. S2. Detailed staining protocols have been previously described (2), with some modifications as the following describes. Briefly, spleen and liver cells were depleted of murine cells using murine CD45 magnetic bead negative selection (Miltenyi). Human NK cells were isolated by magnetic bead negative selection using the NK isolation kit (Miltenyi). Samples were bar-coded using human CD45 live barcoding, as described, to ensure uniformity of staining (58). Bar-coded samples were resuspended in 25 mM cisplatin (Enzo Life Sciences) for 1 min and then quenched with 100% FBS. Cells were stained for 30 min at 4°C, fixed (BD FACS Lyse), permeabilized (BD FACS Perm II), and stained with intracellular antibodies for 45 min at 4°C. Cells were resuspended overnight in iridium intercalator (Fluidigm) in 2% paraformaldehyde in PBS and then washed once with PBS and thrice with Milli-Q water immediately before acquisition on a Helios mass cytometer (Fluidigm).

Mass cytometry data analysis
Samples were manually debar-coded using Boolean combination gates in FlowJo v10.1r3. To ensure the purity of NK cells, serial negative gating was performed before analysis, as shown in fig. S2. Analyses for Figs. 1 and 3 were conducted in the open-source statistical package R. viSNE was implemented on gated NK in Cytobank, as described by Amir et al. (59). Mean intensity values were calculated using FlowJo v10.1r3 and heatmaps were created using GraphPad Prism v7.0.

Research ethics approval for VZV glycoprotein skin test challenge
This work was approved by the Ethics Committee of the Royal Free Hospital. Healthy persons who had a history of childhood chickenpox infection (n = 10; median age, 32.9 years; age range, 22 to 85 years; six males and four females) were recruited for the study. All volunteers provided written informed consent, and study procedures were performed in accordance with the principles of the Declaration of Helsinki.

Participant exclusion criteria
Individuals with history of neoplasia, immunosuppressive disorders, or inflammatory skin disorders were excluded from this investigation. Furthermore, we excluded individuals with comorbidities that are associated with significant internal organ or immune dysfunction including heart failure, severe chronic obstructive pulmonary disease, diabetes mellitus, and rheumatoid arthritis and individuals on immunosuppressive regimes for the treatment of autoimmune or chronic inflammatory diseases. We did not exclude volunteers with a history of uncomplicated hypertension or hypercholesterolemia because this would have prevented most of the aging volunteers from participating in this study. Individuals who had received Zostavax vaccinations or had a history of shingles were also excluded from this study.

Skin tests
DTH responses were induced by intradermal injection of 0.5 ml of VZV antigen. VZV-STA contains viral glycoproteins prepared from the culture fluid of the attenuated VZV Oka parental strain–infected MRC-5 cells (60, 61). VZV antigen (BIKEN, The Research Foundation for Microbial Diseases of Osaka University, Japan) was injected intradermally into sun-unexposed skin of the medial proximal volar forearm as per the manufacturer’s instructions. Baseline skin erythema was determined as the mean of three measurements using the Derma-Spectrometer (Cortex Technology, Hadsund, Denmark). Skin erythema index (EI), size of induration, and palpability were also recorded 3 days after VZV skin challenge, and the change in skin EI was calculated by subtracting the baseline from the VZV challenge measurement. The induration size was determined by calculating the mean of two measured perpendicular planes. Induration, palpability, and the change in erythema from baseline were measured on day 3 after VZV skin challenge to generate a clinical score (0 to 10).

Preparation of suction blister cells and PBMC preparation
To induce skin suction blisters over the site of VZV skin test DTH, the dermis was split from the epidermis by the application of a negative pressure of 25 to 40 kPa (200 to 300 mmHg) below atmospheric pressure, via a suction chamber for 2 to 4 hours using a clinical suction pump (VP25; Eschmann), until a unicellular blister measuring 10 to 15 mm in diameter was formed. Blister fluid was aspirated 16 to 20 hours after blisters were formed using a sterile 23-gauge needle and 2-ml syringe (Tycos Healthcare UK Ltd., Gosport, UK; Fig. 1, I and J) and microcentrifuged at 650 × g for 4 min to pellet the blister fluid–resident cells. The pellet was resuspended in complete RPMI 1640 medium (Life Technologies) containing 10% human AB serum, penicillin (100 U/ml), streptomycin (100 μg/ml), and 2 mM l-glutamine (all were obtained from Sigma-Aldrich). Heparinized blood was collected at the time of blister aspiration for PBMC isolation. PBMCs were prepared by density centrifugation on Ficoll-Paque (Amersham Biosciences) and resuspended in complete medium. Erythrocyte and leukocyte numbers were quantified using a hemocytometer, and viability was assessed by trypan blue exclusion.

Flow cytometric analysis
Multiparameter analysis of PBMC and blister NK cell phenotype was performed on an Aria II (BD Biosciences). PBMCs and blister cells were stained with different combinations of antibodies specific to human CD3 (UCHT1; BioLegend), CD8 (HT18a; BioLegend), CD16 (3G8; BioLegend), CD45 (HI30; BioLegend), CD56 (MEM-188; BioLegend), CD62L (DREG-56; BioLegend), CD69 (FN50; BioLegend), CD107a (H4A3; BioLegend), NKG2D (1D11; BioLegend), CXCXR6 (K041E5; BioLegend), T-bet (04-46; BD), and Eomes (WD1928; Thermo Fisher Scientific). All surface staining was performed for 30 min on ice after previous incubation of cells with human Fc Block and murine Fc Block (BD) for 10 min on ice. After surface staining, cells were fixed and permeabilized using the Foxp3 Transcription Factor Fixation/Permeabilization Concentrate and Diluent Kit (eBiosciences, Thermo Fisher Scientific), as per the manufacturer’s protocol, and stained for the expression of the transcription factors T-bet and Eomes. FMO control stains were performed using PBMC to verify the staining specificity and as a guide for setting markers to delineate positive and negative populations. Gating was set on the live lymphocyte population using forward- and side-scatter profiles to include lymphocytic blasts, followed by single-cell gating using forward- and side-scatter heights and widths, before identification of hematopoietic cells using human CD45 staining.
Fig. S1. Reconstitution efficiency of BLT mice.

Fig. S4. Trans-presented human IL-15 expands NK cell numbers in spleens and livers of BLT mice.

Fig. S5. Pre- and post-sort analyses of liver NK cells from HIV-Env–vaccinated BLT mice sorted for killing assays shown in Fig. 4.

Table S1. Reagents used for cell surface and intracellular staining for CyTOF analysis.

Table S2. Raw data (Excel).

REFERENCES AND NOTES


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Human natural killer cells mediate adaptive immunity to viral antigens

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Recall responses by human NK cells

One of the traditional dividing lines separating innate and adaptive immunity was the restriction of immune memory to adaptive immune cells. This paradigm has been challenged by accumulating evidence that memory responses can be evoked in natural killer (NK) cells from experimental animals, including mice and nonhuman primates. To determine whether human NK cells also exhibit memory responses, Nikzad et al. analyzed NK cells recovered from humanized mice or NK cells found in the viral antigen-challenged skin of adult volunteers who had chickenpox as children. Antigen-specific recall responses by human NK cells were observed in both experimental systems. These findings suggest that human NK memory responses contribute to acquired host protection after either natural infections or vaccine administration.