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Adipocyte IL-15 Regulates Local and Systemic NK Cell Development

Yae-Huei Liou,*† Szu-Wen Wang‡ Chin-Ling Chang,‡ Po-Lin Huang,‡ Mau-Sheng Hou‡
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NK cell development and homeostasis require IL-15 produced by both hematopoietic and parenchymal cells. Certain hematopoietic IL-15 sources, such as macrophages and dendritic cells, are known, whereas the source of parenchymal IL-15 remains elusive. Using two types of adipocyte-specific Il15−/− mice, we identified adipocytes as a parenchymal IL-15 source that supported NK cell development nonredundantly. Both adipocyte-specific Il15−/− mice showed reduced IL-15 production specifically in the adipose tissue but impaired NK cell development in the spleen and liver in addition to the adipose tissue. We also found that the adipose tissue harbored NK progenitors as other niches (e.g., spleen) for NK cell development, and that NK cells derived from transplanted adipose tissue populated the recipient’s spleen and liver. These findings suggest that adipocyte IL-15 contributes to systemic NK cell development by supporting NK cell development in the adipose tissue, which serves as a source of NK cells for other organs. The Journal of Immunology, 2014, 193: 000–000.

Natural killer cells are critical in the innate and adaptive immunity against viral infection and tumor (1, 2). Upon encountering virus-infected or transformed cells, NK cells directly kill the target cells and produce cytokines without prior activation. Stimulated NK cells produce abundant IFN-γ in the early phase of an immune response, which suppresses virus propagation (3) and shapes T cell responses toward the Th1 type (4) that is efficient against intracellular pathogens and tumors. Recent studies demonstrated the development of NK cell memory in response to CMV infection in mice and humans (5, 6).

NK cells originate from hematopoietic stem cells in the bone marrow (BM) and differentiate through the common lymphoid progenitor stage. The common lymphoid progenitors give rise to prepro-NK cells that commit to the NK cell lineage and differentiate into NK progenitors (NKPs) (7). NKPs had been defined as Lin−CD122NK1.1+DX5− (8) cells, which were later found to be heterogeneous and refined as Lin−2B4−CD27+Fli3−CD122+NKGD2NK1.1− (7, 9). NKPs differentiate into immature NK cells (NK1.1+DX5−) and then to mature NK cells (NK1.1+DX5+) (10). The expression of CD27 and CD11b also define three post-NKP differentiation stages, from CD27+CD11b− to CD27+CD11b+ and then to CD27−CD11b− (11, 12); the downregulation of CD27 and upregulation of CD11b coincide with DX5 expression. During post-NKP differentiation, NK cells acquire activating and inhibitory NKRs (13–15). The interaction between the inhibitory NKR and self-MHC class I molecules endows the developing NK cells with cytotoxicity and IFN-γ productivity, a process termed NK education (16, 17). NKPs have been found in the BM, spleen (SP), lymphoid node, fetal thymus, and fetal liver (LV) (8, 18–20), whereas the post-NKP cells at various differentiation stages are distributed throughout lymphoid and nonlymphoid tissues (21–27).

NK cell development and homeostasis in the post-NKP stages require IL-15–triggered signals (18, 28). Expression of IL-15Rβ (CD122) at the NKP stage endows NK cells with the ability to use IL-15 (8). IL-15 and IL-15Rα, the private high-affinity receptor, form a complex in the endoplasmic reticulum and are transported to, and displayed on, the cytoplasmic membrane (29). Some complexes on the cell surface are cleaved into soluble forms (30). The membrane-bound IL-15/IL-15Rα complex on a cell triggers signaling through the IL-15Rβ and γc on a neighboring cell, a mode of usage termed trans-presentation (31, 32). The absence of IL-15 or IL-15Rα results in severe impairment in NK cell maturation, NKR and effector function acquisition, and homeostasis (33–36).

IL-15 and IL-15Rα expressed by both hematopoietic cells and radiation-resistant accessory cells (parenchymal cells) contribute to NK cell development (37, 38). Macrophage IL-15 and dendritic cell (DC) IL-15 contribute to the hematopoietic IL-15 source that supports NK cell development (39, 40), whereas little is known about the parenchymal IL-15 source involved in NK cell development. In this article, we report that parenchymal IL-15 preferentially affects NK cell homeostasis in the epithymal fat pad (EPI), a white adipose tissue. This observation led to the subsequent findings of a supportive function for adipocyte IL-15 in NK cell homeostasis and development within the EPI and in the distal SP and LV. We also found that the EPI harbors NKPs and post-NKP cells that transplanted intact EPI gave rise to NK cells that populated the recipients’ SP and LV. These results suggest that adipocyte IL-15 supports NK cell development in the adipose tissue, which serves as a source of NK cells for other peripheral organs.

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Abbreviations used in this article: BAT, brown adipose tissue; BM, bone marrow; EPI, epidymal fat pad; ES, embryonic stem; KLRG1, killer lectin-like receptor G1; KO, knockout; LV, liver; MUS, gastrocnemius muscle; NKP, NK precursor; RCF, relative centrifugal force; SP, spleen; SVF, stromal vascular fraction; WT, wild-type.
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Materials and Methods

**Mice**

C57BL/6J (B6/J), B6.SJL-Prepcre-Pepckcre/ByJ (CD45.1*B6/J), B6.Cg-Tg (ACTFLPfpk205Dym/J (FLPe), C57BL/6J-Ly5.2-Tac2J (Ly5.2-Tac), B6.Cg-Tg (Fabp4cre/Rev1) (ap2cre), B6.Cg-Tg (Acta1-cre)79meJ (Acta1-cre), C57BL/6J-Tg (Igdx-cx-EgfFp907AehC3 (Cd11c-cre), B6.129P2-Ly5.2m1065J (Ly5mcre), and B6.FVB-Tg(Adipoq-cre)Evd9J (Adipoq-cre) mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and bred in the animal facility of the Institute of Molecular Biology, Academia Sinica. C57BL/6JNTac2J (Ly5.2^+^) mice were generated in our laboratory and backcrossed to B6/J mice for 16 generations. Il15^flox/flox (Il15^flox^) mice were generated in our laboratory and backcrossed to B6/J mice for at least five generations. ap2^IRES-Il15, Ap2^IRES-Il15^*, Cd11c-Il15^*, Ly5m-Il15^*, Adipoq/Cd11c-Il15^*, and Adipoq/Lysm-Il15^* mice were generated by breeding C57BL/6J mice with fluorochrome or biotin were used: CD3 (2C11), CD8 (53-6.7), CD4 (145-2C11), CD69 (H1.2F3), CD19 (6D5), NK1.1 (PK136), NKp46 (29A1.4), CD49b (DX5), CD45 (30F11), and CD45.1 (A20), CD45.2 (104), NKP2D (CX5), NKP2G6 (161i), CD27 (LC74P), killer lectin-like receptor G1 (KLRG1), 2F1, CD44 (IM7), CD122 (5H4), CD11c (N418), TER119 (TER119), CD4 (GK.1), CD3 (503.3), CD7 (9D4), CD91 (18d3), CD11b (M170), Ly49a (YE1/148.106), Ly49q (4D11), Ly49d (4E5), Ly49c1 (56f), Ly49h (3D10), CD49a (H1a1), CD11a (J44), CD11b (1D4B), IFN-γ (XMG1.2), and CD127 (A573) (purchased from ebioscience, San Diego, CA; BioLegend, San Diego, CA; R&D Systems, Minneapolis, MN, and BD Biosciences, or purified from hybridoma supernatant in our laboratory).

**BM chimera**

BM cells isolated from the tibia and femur of CD45.1^+^ mice were depleted of T and NK cells by complement-mediated lysis with anti-Thy1 (J1) and anti-NK1.1 (PK136) mAbs. BM chimeras were generated by i.v. injection of the T- and NK-depleted BM cells (1 × 10^5^) into lethally irradiated (1000 rad, [125^I]Cs source) CD45.2^+^ recipient mice. Chimeras were analyzed 9 wk later. CD45.1^+^ cells constituted 90-98% of live cells in the BM, SP, and LV and ~50% of live cells in the EPI stromal vascular fraction (SVF) that contains 60% of CD45^+^ cells.

**Isolation of adipocytes and SVF**

EPI was cut into pieces and digested with type IV collagenase alone or type IV collagenase plus type VII collagenase at a 1:1 ratio (0.5 mg/100 mg fat/ml KBH8 buffer; Sigma-Aldrich, St. Louis, MO) for 1 h at 37°C. The digested sample was passed through a 70-μm nylon mesh and centrifuged for 5 min at 500 relative centrifugal force (RCF) at room temperature. Adipocytes were in the floating portion, whereas the SVF cells were in the pellet. RBCs in the SVF were lysed with ACK buffer (NH4Cl, 8024 mg/l; KHCO3, 1001 mg/l; EDTA-Na2, 2H2O, 37.22 mg/l).

**Isolation of intrahepatic lymphocytes**

Mice were euthanized and perfused with 3 ml PBS and 7.5 ml digestion buffer (HBSS with 2% FBS, 10 mM HEPES, and 0.002% type IV collagenase) via the portal vein. The liver was excised, cut into pieces, pressed with the barrel of a 3-ml syringe, and filtered through 100-μm cell strainers (BD Biosciences). Total intrahepatic lymphocytes were collected by centrifugation at 630 RCF for 10 min, resuspended in 40% isotonic Percoll, and then pelleted again at 800 RCF for 20 min. RBCs in the intrahepatic lymphocyte preparation were lysed using ACK buffer.

**IFN-γ production and degranulation (CD107a exposure) assay**

Splenocytes were incubated in the absence or presence of plate-bound anti-NK1.1 mAb (PK136; 20 μg/ml), IL-12 (10 ng/ml plus IL-18 (50 ng/ml), or PMA (250 ng/ml) plus ionophore (A23187; 1 μg/ml) for 5 h at 37°C. Brefeldin A (10 μg/ml) was added for the last 4 h of culturing. The culture medium contained 5 μg/ml anti-CD107a mAb, 6 μg/ml monensin (Sigma-Aldrich), and 50 μM marine ril-2 (R&D). Stimulated cells were harvested and stained with specific mAbs for the identification of NK cells (CD19^+^ TCR^+^ NKp46^+^ DX5^+^). IFN-γ production was detected by intracellular staining with anti–IFN-γ mAb (XMG1.2) or its isotype-control Ab.

**Adoptive transfer of SVF cells**

CD45.2^+^ SVF cells prepared from I15^+/^ mice (CD45.2^+^) mice were injected i.v. into sublethally irradiated (100 rad) CD45.1^+^ B6/J mice (3 × 10^5^ SVF cells/recipient). Donor SVF-derived NK cells in the recipients’ SP and LV were analyzed by flow cytometry at 1, 2, and 3 wk after transfer.

**EPI transplantation**

EPI was excised from CD45.1^+^ B6/J mice (two lobes per mouse), rinsed with PBS, and cut into cut pieces per lobes. CD45.2^+^ B6/J mice were sublethally irradiated, anesthetized with Avertin (Sigma-Aldrich), and transplanted s.c. with donor EPI pieces (two original lobes/recipient). Three weeks later, the donor EPI-derived NK cells in the recipients’ SP and LV were analyzed by flow cytometry.

**Quantitative real-time PCR**

SP, LV, gastrocnemius muscle (MUS), brown adipose tissue (BAT), EPI, and EPI adipocytes were quick-frozen by liquid nitrogen. Each BM sample was prepared from a femur by flushing with TRIZol reagent. Total RNA was extracted by TRIZol reagent and reverse transcribed using Superscript III Reverse Transcriptase (Life Technology Invitrogen, Carlsbad, CA). Quantitative real-time PCR was performed using an Applied Biosystems 7500. The gene expression was normalized to cyclophilin A and analyzed by ABI Prism 7500 SDS software (Life Technology Applied Biosystems). The primer sequences were as follows: cyclophilin A: 5'-GAGCTGTGTT-GCAGACAAAGTC-3' (sense) and 5'-CCTCGTTGCTATGACTACGGAC-3' (antisense) and Il15: 5'-TCTCCCTAAATACGATATAGCA-3' (antisense) and 5'-TTGGACACTTGAGTAACTGAC-3' (antisense).

**Measurement of IL-15/LIL-15Ra complex**

Tissue and cell lysates were prepared by homogenization with lysis buffer (20 mM Tris-HCl [pH 7.5], 150 mM NaCl, 1 mM Na2EDTA, 1 mM EGTA, 1% Triton) containing protease inhibitor mixture (Roche Applied Science, Mannheim, Germany). Each BM sample was prepared from a tibia by flushing with lysis buffer. Plasma was prepared from the blood obtained by cardiac puncture using an EDTA blood collection tube (BD Biosciences). The amount of IL-15/LIL-15Ra complex was measured using the mouse IL-15/LIL-15Ra Complex ELISA Kit (ebioscience).

**Statistical analysis**

All results were analyzed by the unpaired, two-tailed Student t test using GraphPad Prism 5 (GraphPad, San Diego, CA). The error bars in the bar graphs and dot plots represent SD and SEM, respectively.

**Results**

Parenchymal IL-15 preferentially regulates adipose NK cell homeostasis

To examine the contribution of hematopoietic IL-15 versus parenchymal IL-15 to NK cell homeostasis, we generated constructed
radiation BM chimeras with various combinations of Il15+/+ (wild-type [WT]) and Il15−/− (knockout [KO]) BM cells and recipients. Comparing KO→WT and WT→WT chimeras, hematopoietic IL-15 deficiency caused a 60–75% NK cell reduction in the BM, SP, and LV, as well as a 33% reduction in the EPI (Fig. 1). Comparing WT→KO and KO→KO chimeras, hematopoietic IL-15 restored NK cells in a KO environment to >80% of the WT→WT level in the BM and SP but only to 24% of the WT→WT level in the LV and EPI. These results reveal a difference in the dependency on hematopoietic IL-15 for NK cell homeostasis among different organs (i.e., BM, SP, and LV depend more on hematopoietic IL-15 in comparison with EPI). Also, the lower NK cell reduction in EPI of KO→WT chimeras compared with the other organs suggests a more prominent role for parenchymal IL-15 in supporting NK cell homeostasis. By comparing WT→KO and WT→WT chimeras, we found that the deficiency in the recipient IL-15 did not change NK cell homeostasis in the BM; however, it resulted in a 20% reduction in SP and a 76% reduction in LV and EPI. This result indicates that, even in the presence of hematopoietic IL-15, parenchymal IL-15 deficiency resulted in a severe reduction in the NK cells in LV and EPI. Taken together, these results indicate that hematopoietic IL-15 preferentially affected NK cell homeostasis in BM and SP, whereas parenchymal IL-15 preferentially affected NK cell homeostasis in the adipose tissue. Both sources of IL-15 affected hepatic NK cell homeostasis equally.

Generation of adipocyte-specific Il15−/− mice

Because adipocytes constitute the main parenchyma of the adipose tissue and are capable of producing IL-15 (41, 42), the preferential effect of parenchymal IL-15 on EPI NK cell homeostasis prompted us to investigate the role of adipocyte IL-15 in NK cell development. Thus, we generated adipocyte-specific Il15-KO mice by producing Il15flox/flox (Il15f/f) mice (Materials and Methods, Fig. 2A) and crossing them to mice transgenic for cre driven by the fatty acid binding protein 4 promoter (also known as aP2-cre) or the adiponectin promoter (also known as Adipoq-cre). AP2 is primarily expressed by adipocyte progenitors and upregulated along differentiation of adipocytes (43–45). Because of the high expression of AP2 by mature adipocytes, aP2-cre mice have been used to generate various adipocyte-specific gene–KO mice (46, 47). However, AP2 protein is also expressed by cardiomyocytes, DCs, and macrophages (48–51), whereas adiponectin is adipocyte specific (52). Previous studies revealed a range of sensitivity to recombination mediated by either adipocyte-specific cre for various floxed gene loci (47); therefore, we generated and characterized aP2-Il15f/f mice first and then confirmed with Adipoq-Il15f/f mice.

IL-15 RNA levels vary in different organs of Il15f/f mice (Supplemental Fig. 1). Compared with the Il15f/f littermates, both aP2-Il15f/f and Adipoq-Il15f/f mice had reduced IL-15 RNA in the interscapular BAT on the dorsal side (53) and in EPI, but not in

**FIGURE 1.** Parenchymal IL-15 preferentially regulates adipose NK cell homeostasis. Cells prepared from BM, SP, LV, and EPI SVF of indicated BM chimeras were analyzed by flow cytometry. NK cells (NK1.1+ TCRβ−) were analyzed in live CD19− cells gated from CD45.1+ lymphocyte-size like cells (upper panel). Comparison of NK cell percentage in CD45.1+ live lymphocyte-size like cells among the four types of BM chimeras was done by normalization to Il15+/+→Il15+/+ chimera (mean ± SD, n = 4–6/group) (lower panel). The number above each bar indicates the percentage of reduction. Data are representative of two independent experiments. *p < 0.05, **p < 0.005, ***p < 0.0001.
FIGURE 2. Generation of adipocyte-specific Il15−/− mice. (A) Schematic diagram of Il15 WT, gene targeted (GT), and flox allele. The Il15 GT ES cell clone was justified by the approximate 2:1 ratio of PCR product of Il15 (primer e and d) but the equal ratio of control Inpp4b and Tbc1d9. (B) Expression of IL-15 mRNA in BM, SP, LV, EPI, and EPI adipocytes. RQ value represents expression level relative to the Il15f/f littermates (n = 6–8/group). Data are combined from three independent experiments. (C) Amount of IL-15/IL-15Ra complex in tissues and EPI adipocyte lysates (n = 6–8/group). Data were combined from three independent experiments. (D) Amount of plasma IL-15/IL-15Ra complex (n = 22–31/group). Data are (Figure legend continues)
FIGURE 3. Adipocyte-specific II15<sup>−/−</sup> mice exhibit impaired NK cell homeostasis. NK cells in each organ of the indicated mouse type were analyzed by flow cytometry. BM cells were isolated from a tibia and a femur. In dot plots, NK cells (circled) are NK1.1<sup>+</sup>TCR<sup>β</sup><sup>+</sup> cells in CD19<sup>−</sup> cells gated from live lymphocyte-size cells. Data for NK cell percentage in total live cells or live lymphocyte-size cells and for NK cell number/organ are compiled (mean ± SEM). Each symbol represents one mouse. The number above the KO data group indicates the percentage of reduction compared with II15<sup>±/±</sup> littermates. Data are combined from at least three independent experiments. *p < 0.05, **p < 0.005, ***p < 0.0001.

combined from at least three independent experiments. In (B)–(D), each bar represents mean ± SD of samples from aP2-II15<sup>−/−</sup> mice, Adipoq-II15<sup>−/−</sup> mice, or II15<sup>±/±</sup> littermates. The number above the bar indicates the percentage of reduction compared with the littermates. *p < 0.05, **p < 0.005, ***p < 0.0001.
FIGURE 4. Adipoq-Il15ff mice exhibit impaired NK cell maturation. (A) Flow cytometry analysis of NK subsets defined by the expression of DX5 and CD49a in total NK cells (CD19−NK1.1−TCRβ+) from the indicated organs of Adipoq-Il15ff mice in comparison with their littermates. BM cells were isolated from a tibia and a femur. The cell number of each NK subset is presented as mean ± SEM. (B) Expression of CD27 and CD11b by NK subsets defined in (A). The cell number in each maturation stage is presented as mean ± SEM. Each symbol represents one mouse. The number above each cluster indicates the percentage of reduction compared with Il15ff littermates. Data are combined from at least three independent experiments. *p < 0.05, **p < 0.005, ***p < 0.0001.
NK cells in Adipoq-II15f/f mice exhibit impaired acquisition of Ly49C/I, KLRG1, and effector functions. Flow cytometric analysis of Ly49C/I (A) and KLRG1 (B) expression in live CD19- NK1.1+TCRβ2+ cells from the indicated organs from Adipoq-II15f/f mice in comparison with their littermates. BM cells were isolated from a tibia and a femur. The NK cell number/organ is presented as mean ± SEM. Each symbol represents one mouse. The number above each cluster indicates the percentage of reduction in the adipocyte-specific Il152/2 sample compared with the sample from Il15f/f littermates. Data are combined from at least three independent experiments. (C) Degranulation and IFN-γ production by NK cells. Splenic NK cells were stimulated with PBS, plate-bound anti-NK1.1 mAb (α-NK1.1), IL-12 plus IL-18 (IL-12/18), or PMA plus ionophore (P/I), as described in Materials and Methods. Cell surface CD107a and intracellular IFN-γ were analyzed in NK cells (CD19- TCRβ- NKp46+DX5+CD49a-) by flow cytometry.

FIGURE 5. NK cells in Adipoq-II15f/f mice exhibit impaired acquisition of Ly49C/I, KLRG1, and effector functions. Flow cytometric analysis of Ly49C/I (A) and KLRG1 (B) expression in live CD19- NK1.1+TCRβ2+ cells from the indicated organs from Adipoq-II15f/f mice in comparison with their littermates. BM cells were isolated from a tibia and a femur. The NK cell number/organ is presented as mean ± SEM. Each symbol represents one mouse. The number above each cluster indicates the percentage of reduction in the adipocyte-specific Il152/2 sample compared with the sample from Il15f/f littermates. Data are combined from at least three independent experiments. (C) Degranulation and IFN-γ production by NK cells. Splenic NK cells were stimulated with PBS, plate-bound anti-NK1.1 mAb (α-NK1.1), IL-12 plus IL-18 (IL-12/18), or PMA plus ionophore (P/I), as described in Materials and Methods. Cell surface CD107a and intracellular IFN-γ were analyzed in NK cells (CD19- TCRβ- NKp46+DX5+CD49a-) by flow cytometry.
BM, LV, SP, or MUS (Fig. 2B, left panels). Consistently, EPI adipocytes of either KO mouse showed reduced IL-15 RNA levels (Fig. 2B, right panels). We next examined IL-15 expression at the protein level and found that the amount of IL-15/IL-15Rα complex decreased in BAT and EPI lysates, but not in BM, LV, SP, or MUS lysates, prepared from aP2-Il15f/f and Adipoq-Il15f/f mice in comparison with those prepared from Il15f/f littermates (Fig. 2C, left panels). Consistently, EPI adipocyte lysates prepared from either KO mouse showed reduced levels of IL-15/IL-15Rα complex (Fig. 2C, right panels). Despite many similarities in IL-15 expression between the two adipocyte-specific Il152/2 mice, the level of plasma IL-15/IL-15Rα complex decreased by 13% in aP2-Il15f/f mice but was normal in Adipoq-Il15f/f mice (Fig. 2D). Taken together, these results indicate that adipocyte IL-15 was the primary deficiency in both aP2-Il15f/f and Adipoq-Il15f/f mice.

Adipocyte-specific Il152/2 mice exhibit impaired NK cell homeostasis

We next examined whether adipocyte IL-15 affects NK cell homeostasis by comparing the adipocyte-specific Il152/2 mice with the Il15f/f littermates, who had a similar plasma IL-15/IL-15Rα level and NK cell number as WT mice (Supplemental Fig. 2). Both aP2-Il15f/f and Adipoq-Il15f/f mice showed reduced NK cell percentages and numbers in SP, LV, and EPI but not in BM (Fig. 3). This result indicates that adipocyte IL-15 supported NK cell homeostasis in EPI, as well as SP and LV, without affecting that in BM. Moreover, the degree of NK cell reduction was comparable between EPI of the two KO mice but was more severe in SP and LV of aP2-Il15f/f mice. This result is consistent with the idea that AP2 is also expressed by certain type(s) of cells in nonadipose tissue, whose IL-15 deficiency enhanced the NK cell reduction in SP and LV of aP2-Il15f/f mice. Thus, we subsequently focused on data obtained from Adipoq-Il15f/f mice.

Adipoq-Il15f/f mice exhibit impaired NK cell maturation

Recent studies demonstrated the presence of DX5+CD49a+ memory NK cells in LV (54); therefore, we analyzed NK cell subsets defined by DX5 and CD49a expression. Approximately 90% of BM and splenic NK cells in the Il15f/f littermates were the conventional DX5+CD49a+ NK cells, which were reduced by 30% in SP, but not in BM, of Adipoq-Il15f/f mice. LV of Il15f/f mice harbored DX5+CD49a+ and DX52CD49a+ NK cells at a 2:1 ratio, as reported previously; both NK subsets were reduced by 30% in Adipoq-Il15f/f mice. In EPI, half of the NK cells consisted of equally proportioned DX5+CD49a+ and DX52CD49a+ subsets; the other half was DX52CD49a+, which has not been reported previously. All three EPI NK subsets were reduced by 33–48% in Adipoq-Il15f/f mice (Fig. 4A). The reduction in these NK subsets also was observed in aP2-Il15f/f mice (Supplemental Fig. 3A).
FIGURE 7. Adipose tissue harbors NKPs and provides NK cells to recipient’s SP and LV upon transplantation. (A) Analysis of NKPs. CD45.2+CD3-CD19-CD11b+ cells were gated from total live cells of each organ from B6/J mice. The cells were further gated for NK1.1+, CD27+, CD122+, and NKG2D+ and examined for the expression of CD127. The number of NKPs (CD45.2+CD3-CD19-CD11b+NK1.1+CD27+CD122+NKG2D+) in each organ is presented (mean ± SEM). BM cells were isolated from a tibia and a femur. Each symbol represents one mouse. Data are representative of two independent experiments. (B) Adoptive transfer of Il15−/− (CD45.2+) EPI SVF cells into sublethally irradiated Il15+/+ (CD45.1+) recipients. The percentage of donor (CD45.2+) cells in the SP and LV CD19−NK1.1+TCRb+ NK cells was determined before (Day 0) and after transplantation at the indicated time points (left panels). The data are mean ± SEM; each symbol represents one recipient. The expression of CD27 and CD11b by the CD45.2+ donor-derived NK cells was analyzed at the indicated time points (right panels). The data are mean ± SEM; each symbol represents the percentage of the indicated NK subset in CD45.2+ NK cells. This experiment was performed independently three times with one or two mice for (Figure legend continues)
Taken together, these results indicate that a deficiency in adipocyte IL-15 caused a reduction in NK subsets defined by CD49a and DX5 expression in EPI, SP, and LV but not in BM.

We further examined NK cell maturation in each DX5/CD49a-defined NK subset according to the three maturation stages: CD27+CD11b+ (stage 1), CD27+CD11b− (stage 2), and CD27−CD11b+ (stage 3). We found that the overall distribution of these three stages of cells in BM, SP, LV, and EPI was largely similar between Adipoq-Iil15−/− and Il15−/− mice (Fig. 4B). The conventional DX5+CD49a+ NK cells were enriched in stage 2 and stage 3, whereas the DX5+CD49a− NK cells were enriched in stage 1 cells. The novel DX5+CD49a+ subset in EPI contained >90% of stage 1 and stage 2 cells, which were present at an approximately equal ratio. In the DX5+CD49a− subset of SP, LV, and EPI, stage 3 cells were decreased in Adipoq-Iil15−/− mice compared with Il15−/− litters (Fig. 4B). In the DX5+CD49a− subset of SP and LV and EPI, stage 2 cells were decreased in both organs, whereas stage 1 cells were decreased only in EPI of Adipoq-Iil15−/− mice. In the DX5+CD49a− subset of EPI, stage 1 and stage 2 cells were decreased in Adipoq-Iil15−/− mice, although there was no reduction in all examined BM NK subsets in Adipoq-Iil15−/− mice. The reduction in NK subsets also was found in ap2-Iil15−/− mice (Supplemental Fig. 3B). Taken together, the deficiency in adipocyte IL-15 resulted in the decrease in NK cells at various differentiation stages in EPI, SP, and LV.

We next examined the acquisition of NKRs and the terminal differentiation marker, KLRG1 (55). We found a reduction in Ly49C/I− and KLRG1− NK cells in SP, LV, and EPI, but not BM, of Adipoq-Iil15−/− mice compared with the Il15−/− litters (Fig. 5A, 5B). This phenotype also was present in ap2-Iil15−/− mice (Supplemental Fig. 3C). These results indicate a positive role for adipocyte IL-15 in the acquisition of Ly49C/I and KLRG1 by NK cells.

Lastly, we examined the effector function of splenic NK cells, because >80% of them are stage 2 and stage 3 cells that display greater effector function than stage 1 cells (12). Degranulation and IFN-γ production by splenic DX5+CD49a+ NK cells were examined after stimulation with immobilized anti-NK1.1Ab, IL-12 plus IL-18, or PMN plus ionophore (56–58). We found a decrease in NK1.1-triggered CD107a−IFN-γ− NK and IL-12/18-induced CD107a−IFN-γ+ NK cells in Adipoq-Iil15−/− mice compared with the Il15−/− counterparts (Fig. 5C). The proportion of the NK1.1-triggered CD107a−IFN-γ− NK subset also decreased in ap2-Iil15−/− mice (Supplemental Fig. 3D). These results suggest a positive role for adipocyte IL-15 in the effector function of splenic NK cells. In summary, adipocyte IL-15 positively regulated local and systemic NK cell homeostasis and development and, possibly, effector function.

Adipocyte IL-15 plays a nonredundant role from macrophage or DC IL-15 in support of NK cell homeostasis in LV and EPI

Deficiency in either macrophage or DC IL-15 impairs NK cell homeostasis (39). To determine whether adipocyte IL-15 and the two hematopoietic IL-15s are functionally redundant, we generated double tissue–specific Il15−/− mice to analyze NK cell homeostasis. The Cdl11c and Lysm promoters are specific for DCs and macrophages, respectively. In SP, there was no additive effect between adipocyte and macrophage IL-15 deficiencies or between adipocyte and DC IL-15 deficiencies with regard to the reduction in NK cell percentage (Fig. 6A) and number (Fig. 6B). In LV, Adipoq/Cdl11c-Iil15−/− mice showed more NK cell reduction than did the two single tissue–specific Il15−/− mice, whereas Adipoq/Lysm-Iil15−/− mice were similar to Lysm-Iil15−/− mice (Fig. 6). Therefore, there was an additive effect between adipocyte and DC IL-15 deficiencies, but not between adipocyte and macrophage IL-15 deficiencies, on LV NK cell reduction. In EPI, both Adipoq/Cdl11c-Iil15−/− and Adipoq/Lysm-Iil15−/− mice showed more severe NK cell reduction than did the single tissue–specific Il15−/− mice (Fig. 6). These results indicate that adipocyte IL-15 played a nonredundant supportive role in local and hepatic NK cell homeostasis.

Adipose tissue harbors NKPs and provides NK cells to recipient’s SP and LV upon transplantation

Adipoq-Iil15−/− mice showed impaired NK cell homeostasis and development in adipose and nonadipose tissues. Considering the normal level of plasma IL-15/IL-15R complex in Adipoq-Iil15−/− mice, we hypothesized that the effect of adipocyte IL-15 deficiency on nonadipose tissues is at least in part contributed by the reduced supply of NK cells from adipose tissue. We first examined whether adipose tissue harbors NKPs like other peripheral niches for NK cell development (e.g., SP and LV). We found that NKPs, defined as CD3+CD19+CD245.2+NK1.1−CD27+CD122+KLRG1−CD127+ cells, (7, 9), were present in EPI, as well as in BM, SP, and LV (Fig. 7A). We next examined whether EPI NKPs give rise to NK cells that populate other organs by adoptive transfer of CD45.2+Il15−/− EPI SVF cells into sublethally irradiated CD45.1+ WT mice, because NK cells do not develop beyond the NKP stage in Il15−/− mice. There was no detectable flow cytometry background of the donor congenic marker, CD45.2, in SP and LV NK cells of irradiated mice before SVF transfer (Fig. 7B, upper left panel day 0). Over a period of 3 wk after transfer, CD45.2+Il15−/− SVF-derived NK cells constituted 0.02–0.1% of NK cells in the recipients’ SP and LV (Fig. 7B, upper left panel Wk 1–3). Analysis of CD27 and CD11b expression by the donor SVF-derived NK cells identified an increase in stage 3 cells at wk 3 after transfer (Fig. 7B, right panels). These results imply that EPI NKPs are able to mature and populate other organs. However, it is not known whether NKPs egress from EPI. Thus, we transplanted CD45.1+ EPI into sublethally irradiated CD45.2+ mice to examine whether EPI provides NK cells to the periphery (59). Little flow cytometry background of the donor congenic marker, CD45.1, was detected in SP and LV lymphocytes of irradiated mice before EPI transplantation (Fig. 7C, Day 0). One week after transplantation, the CD45.1+ donor EPI–derived cells constituted 0.01% of total live lymphocytes in the recipient’s SP and LV (Fig. 7C, Wk 3 left panels). The percentages of donor and recipient NK cells in their own CD45.2+ pool were comparable (Fig. 7C, Wk 3 middle panels). Distinctively, ~80% of donor EPI-derived NK cells were stage 3 NK cells, whereas only 40% of recipient NK cells were at stage 3 (Fig. 7C, Wk 3 right panels). These results suggest that cells in EPI could egress and supply NK cells to the other organs, such as SP and LV. Together with the presence of NK cells at the NKP and post-NKP stages, EPI likely serves as a niche for NK cell development and supply.

Discussion

NK cell development is dependent on IL-15 derived from hematopoietic and parenchymal sources. DC and macrophage IL-15
contribute to the hematopoietic source, whereas the parenchymal IL-15 source remains elusive. This study identified a nonredundant role for adipocyte IL-15 in the support of local, as well as systemic, NK cell development. Moreover, we found that EPI harbored NKPs, and that NK cells derived from transplanted EPI populated the recipient’s SP and LV. Together, these results suggest that adipocyte IL-15 supports the development of adipose NK cells in EPI, which serves as an NK cell source for other organs.

In this study, we used Adipoq-IL15f/f and Adipoq-Il15f/f mice to investigate the function of adipocyte IL-15 in NK cell development. The degrees of IL-15 RNA and IL-15/IL-15R protein reduction in the adipose tissue of both mice are within the range of previous work using either cre transgene to KO other genes (47). However, adipocytes had greater IL-15 reduction in the adipose tissues and plasma blood, whereas Adipoq-Il15f/f mice had greater IL-15 reduction in the adipocytes. Moreover, the level of plasma IL-15/IL15R was normal in Adipoq-Il15f/f mice, but it decreased by 13% in adipocytes. These differences between the two adipocyte-specific Il15−/− mice are consistent with the facts that adiponectin is adipocyte specific, and AP2 is expressed by other cells in addition to adipocytes. Therefore, the more severe IL-15 reduction in adipose tissues and plasma of adipocytes likely reflected IL-15 deficiency in nonadipocyte cells present in adipose and other tissues in the animal. In contrast, the lower adipocyte IL-15 in Adipoq-Il15f/f mice compared with adipocytes indicates that Adipoq-cre is more effective than adipocytes in the generation of adipocyte-specific gene KO. Therefore, we believe that the NK cell phenotypes in the Adipoq-Il15f/f mouse more accurately reveal the function of adipocyte IL-15.

In IL-15–presenting cells provide IL-15 to target cells via trans-presentation (31). Macrophages and DCs, the IL-15 presenters known to affect NK cell biology, exist in various tissues and lymphoid organs and can be distributed throughout the body via circulation (39, 60). In contrast, adipocytes are nonmobile and can only present IL-15 to NK lineage cells within the adipose tissue. We found that EPI resembles other peripheral niches for NK cell development, such as SP and LV, in harboring NKPs, as well as post-NKP cells. Distinct from these niches, NK cell homeostasis in EPI preferentially depends on parenchymal IL-15, presumably adipocyte IL-15. This is supported by the stronger effect of adipocyte IL-15 deficiency on NK cell homeostasis and development in EPI than in SP and LV. These findings are in line with the idea that adipocyte IL-15 acts locally to support NK cell development in the adipose tissue.

In addition to affecting local NK cell development, adipocyte IL-15 deficiency impaired NK cell development and homeostasis in SP and LV. Further, the distal effect of adipocyte IL-15 on LV NK cell homeostasis was nonredundant from DC IL-15. How does adipocyte IL-15 exert a distal effect? The normal plasma IL-15/IL-15R level of Adipoq-Il15f/f mice largely excludes the possibility that the distal effect is mediated by soluble IL-15/IL-15R in the blood. Because adipocyte IL-15 deficiency did not affect NK cell homeostasis and development in the BM, the distal effect of adipocyte IL-15 likely does not involve the BM. We found that NK cells derived from intact EPI graft populated the recipient’s SP and LV; among them, 80% displayed a stage 3 phenotype 3 wk posttransplantation. This finding suggests that adipose NK cells can leave the adipose tissue and populate other organs. Therefore, adipocyte IL-15 is a critical source of IL-15 to support NK cell development within the adipose tissue and, thus, may influence distal NK cell development by affecting NK cell supply from the adipose tissue to other organs. In summary, this study identified adipocyte IL-15 as a critical parenchymal IL-15 source that nonredundantly supports local and systemic NK cell development and suggests that the adipose tissue serves as a NK cell source for other organs.

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Disclosures
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