Molecules and Cells

# Neural-Cadherin Influences the Homing of Terminally Differentiated Memory CD8 T Cells to the Lymph Nodes and Bone Marrow

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**Memory T ($T_M$) cells play an important role in the long-term defense against pathogen reinvasion. However, it is still unclear how these cells receive the crucial signals necessary for their longevity and homeostatic turnover. To understand how $T_M$ cells receive these signals, we infected mice with lymphocytic choriomeningitis virus (LCMV) and examined the expression sites of neural cadherin (N-cadherin) by immunofluorescence microscopy. We found that N-cadherin was expressed in the surroundings of the white pulps of the spleen and medulla of lymph nodes (LNs). Moreover, $T_M$ cells expressing high levels of killer cell lectin-like receptor G1 (KLRG1), a ligand of N-cadherin, were co-localized with N-cadherin$^+$ cells in the spleen but not in LNs. We then blocked N-cadherin *in vivo* to investigate whether it regulates the formation or function of $T_M$ cells. The numbers of CD127$^{hi}$CD62L$^{hi}$ $T_M$ cells in the spleen of memory P14 chimeric mice declined when N-cadherin was blocked during the contraction phase, without functional impairment of these cells. In addition, when CD127$^{lo}$KLRG1$^{hi}$ $T_M$ cells were adoptively transferred into anti–N-cadherin–treated mice compared with control mice, the number of these cells was reduced in the bone marrow and LNs, without functional loss. Taken together, our results suggest that N-cadherin participates in the development of CD127$^{hi}$CD62L$^{hi}$ $T_M$ cells and homing of CD127$^{lo}$KLRG1$^{hi}$ $T_M$ cells to lymphoid organs.**

**Keywords:** homeostasis, homing, memory CD8 T cells, neural cadherin

## INTRODUCTION

The importance of long-term protection against various pathogens, including viruses and bacteria, has been highlighted by the emergence of recent pandemics. Among the many cells participating in the immune responses to these pathogens, memory T ($T_M$) cells are known to mount rapid and robust inflammatory and cytotoxic responses to clear infections (Bevan, 2011; Masopust and Picker, 2012; Sheridan and Lefrançois, 2011). These cells are long-lived and homeostatically proliferate without further stimulation by antigens (Surh and Sprent, 2008; Tanchot et al., 1997). Although the formation of $T_M$ cells is the goal of many vaccine strategies (Ahlers and Belyakov, 2010), the molecular mechanisms of the development and homeostasis of these cells *in vivo* are incompletely defined.

Naïve CD8 T cells activated by cognate antigens develop into multiple subsets of effector T cells to fight against infections (Butcher and Picker, 1996; Nolz et al., 2011). These subsets include CD127$^{lo}$KLRG1$^{hi}$ terminally differentiated effector T cells (TDECs) and CD127$^{hi}$KLRG1$^{lo}$ memory pre-

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cursor effector T cells (MPECs) (Joshi et al., 2007). After the antigen is cleared, these MPECs survive and become $T_M$ cells. $T_M$ cells have been categorized into at least three different subsets based on their surface markers and migratory capacities (Hamann et al., 1997; Mackay et al., 2013; Martin and Badovinac, 2018; Sallusto et al., 1999; Zhang and Lakkis, 2015). Among circulating $T_M$ cells, $CCR7^{hi}CD62L^{hi}$ central memory T ($T_{CM}$) and $CCR7^{lo}CD62L^{lo}$ effector memory T ($T_{EM}$) cells survey lymphoid organs and peripheral tissues, respectively. The third set of $T_M$ cells, resident memory T ($T_{RM}$) cells, are $CD69^{hi}CD103^{hi}$. They do not circulate but instead reside in peripheral tissues (Mueller and Mackay, 2016; Schenkel and Masopust, 2014; Topham and Reilly, 2018). Individual subsets of these $T_M$ cells are differentially localized in the body due to their homing receptor and adhesion molecules, and they utilize different signaling pathways for homeostasis (Hamann et al., 1997; Jiang et al., 2012; Jung et al., 2010; Mackay et al., 2013; Sallusto et al., 1999; Sheridan and Lefrançois, 2011). Once their migratory capacities are altered, their dependencies on certain cytokines are also modified, suggesting that there are specific anatomic locations within tissues for receiving survival signals (Choi et al., 2020; Jung et al., 2016; Park et al., 2020).

Niches, which are specialized microenvironments that provide important signals, including adhesion molecules and cytokines, were first identified as central elements in the development and survival of embryonic and hematopoietic stem cells (HSCs) (Adams and Scadden, 2006; Morrison and Scadden, 2014; Wilson and Trumpp, 2006). Niche cells localized in these microenvironments provide important signaling molecules for stem cells to make fate decisions. In addition, the physical attachment of stem cells to specific niche cells influences the identities of the stem cells (Watt and Hogan, 2000). Taking these observations into account and considering the similarities between the homeostasis of stem cells and $T_M$ cells, we hypothesized that adhesion molecules modulate the homeostasis of $T_M$ cells.

Neural cadherin (N-cadherin) and epithelial cadherin (E-cadherin) are types of cadherins, which are transmembrane or membrane-associated molecules that serve as cell adhesion proteins. In general, cadherins form homogeneous adhesions in a calcium-dependent manner (Alimperti and Andreadis, 2015), but different ligands have been identified as binding partners for cadherins in a heterogeneous manner. These adhesions can induce various biological processes, such as homeostasis and organogenesis (Derycke and Bracke, 2004; Gärtner et al., 2015; Hayashi et al., 2007; Karpowicz et al., 2009; Li et al., 2010; Masai et al., 2003; Matsunaga et al., 1988; Soncin and Ward, 2011). For example, N-cadherin has been shown to maintain quiescence of adult stem cells in their niches, and inhibition of this adhesion molecule led to the activation of stem cells (Goel et al., 2017). In addition to adult stem cells and HSCs, lymphocytes, such as T cells and NK cells, have been shown to express the ligands of cadherins. Among these ligands, KLRG1, a signature marker for TDECs, can bind to N-cadherin, whereas CD103, an important molecule for $T_{RM}$ residency, has been found to adhere to E-cadherin (Tessmer et al., 2007).

These observations led us to hypothesize that cadherins play critical roles in the formation and maintenance of $T_M$ cell subsets in response to acute viral infections. By employing a lymphocytic choriomeningitis virus (LCMV) infectious animal model, we show here that the blockade of N-cadherin altered the formation $CD127^{hi}CD62L^{hi}$ $T_M$ cells in the spleen. Furthermore, the maintenance of $CD127^{lo}KLRG1^{hi}$ $T_M$ cells was modulated in the bone marrow (BM) and lymph nodes (LNs) with the injection of an N-cadherin monoclonal antibody (mAb), suggesting the importance of N-cadherin in the homeostasis of $T_M$ cells. Overall, this study provides another dimension to the understanding of $T_M$ cell development by highlighting the importance of adhesion molecules in specialized microenvironments.

## MATERIALS AND METHODS

### Mice and acute viral infection model

Female C57BL/6 (B6) mice (5 to 6 weeks old) were purchased from OrientBio (Korea). $Ly5.1^+$P14 TCR transgenic mice were obtained from Korea Advancer Institute of Science & Technology (KAIST) (Korea). These mice were maintained and bred under the specific pathogen-free condition. In order to generate P14 chimeric mice, $2.5 \times 10^4$ of $Ly5.1^+P14^+$ cells were adoptively transferred into naïve B6 mice. Naïve B6 or P14 chimeric mice were infected with $2 \times 10^5$ PFU of LCMV-Armstrong strain (LCMV-Arm) via intraperitoneal (i.p). injection. To induce recall response, $2 \times 10^6$ PFU of LCMV-Arm virus was administered via the i.p route. All animal experiments were performed with approval from the Institutional Animal Care and Use Committees at Korea University (KUIACUC 2019-0064).

### *In vivo* blockade of N-cadherin

P14 chimeric mice (> day 30 p.i.) were intraperitoneally treated with 10 μg of anti–N-cadherin mAb (Sigma-Aldrich, USA) diluted in phosphate-buffered saline (PBS; Hyclone, USA) to block N-cadherin *in vivo* during the memory phase or contraction phase.

### Flow cytometry

Isolated lymphocytes were stained with $gp_{33-41}$ tetramer, anti-CD8α, CD45.1 (Ly5.1), CD90.1 (Thy1.1), CD62L, KLRG1, CD127, CD324 (E-cadherin) (Biolegend, USA), or CD325 (N-cadherin) (Miltenyi Biotec., Germany) for 30 min at 4°C. Stained cells were fixed with a fixative containing 2% paraformaldehyde for 10 min at 4°C. The fluorescence intensity of the stained samples was examined by using flow cytometry (BD LSRFortessa™ cell analyzer; BD Biosciences, USA). Collected data were analyzed with FlowJo® software (ver. 10; Tree star Inc., USA).

### Immunofluorescence microscopy

Mice were euthanized at the desired time points and the spleen, LNs, liver and lung tissues were harvested. The tissue segments were immediately washed using PBS and embedded in Tissue-Tek® O.C.T compound (Sakura, Japan) for freezing. These samples were frozen using isopentane with dry ice and then sectioned at a thickness of 8 μm. The sections were air-dried and then fixed in acetone at –20°C for 10

min. Fixed samples were air-dried again and rehydrated using PBS containing 0.05% Tween 20 (PBST). The sections were blocked with a blocking buffer containing 10% normal donkey serum (Jackson ImmunoResearch, USA) and 1% bovine serum albumin (Sigma-Aldrich) for 1 h and then stained with the antibodies against LYVE-1, Ly5.1, B220, CD4, KLRG1, E-cadherin (Biolegend), and N-cadherin (Sigma-Aldrich). APC-conjugated streptavidin was additionally used for the detection of cells attached to a biotinylated antibody. Stained samples were washed three times using PBST and mounted with ProLong™Antifade Mountant (Thermo Fisher Scientific, USA). Sample images were taken using a fluorescence microscope (Axio observer.A1; Carl Zeiss, Germany). The contrast and brightness of the images were compensated and normalized using Photoshop® CS6 (Adobe, USA) and Axio vision (Rel.4.8.2; ZEISS, USA). The number of $T_M$ cells in the T-cell zone were counted using ImageJ software (NIH, USA).

### *In vitro* peptide stimulation and intracellular cytokine staining

Splenocytes were suspended in complete RPMI1640 medium (Hyclone) containing 10% fetal bovine serum (Hyclone) and 1% penicillin/streptomycin (Hyclone) and seeded in a flat-bottom 96-well plate (Corning) at a concentration of $1 \times 10^6$ cells. The cells were treated with brefeldin A (BioLegend) and $gp_{33-41}$ peptide (Genscript, USA) and incubated at 37°C for 6 h. After incubation, the cells were harvested and stained to identify the adoptively transferred P14 cell marker CD90.1 (Thy 1.1) or CD45.1 (Ly5.1). The stained cells were fixed and permeabilized with Cytofix/Cytoperm™solution (Becton Dickinson, USA) at 4°C for 10 min. The fixed cells were stained with anti-TNFα and anti-IFNγ (Biolegend) antibodies diluted in Perm/Wash™buffer (Becton Dickinson) for 1 h at 4°C. After this intracellular staining, cells were washed twice using Perm/Wash™ buffer.

### Statistical analysis

Student's *t*-test, two-way ANOVA, or Wilcoxon signed-rank test were used for statistical analysis. All the error bars in the bar graphs represent mean ± SEM. *P* values < 0.05 represent statistically significant differences in all the experiments.

## RESULTS

### The expression of E-cadherin and N-cadherin in the lymphoid and non-lymphoid organs (NLOs)

HSCs and other adult stem cells receive various forms of niche signals for their homeostasis (Chacón-Martínez et al., 2018; Crane et al., 2017), such as E-cadherin or N-cadherin, can serve as important signals within niches (Chen et al., 2013; Goel et al., 2017)HSCs, $T_M$ cells have been shown to proliferate in the organs homeostatically. Therefore, we hypothesized that microenvironments regulating the homeostasis of $T_M$ cells exist in organs, and these sites provide adhesion molecules as homeostatic signals for $T_M$ cells. First, we investigated whether E-cadherin or N-cadherin is expressed in the lymphoid organs and NLOs of the naïve or memory phase via immunofluorescence microscopy and flow cytometry (Fig. 1A). N-cadherin was expressed in the surroundings of the white pulp of the spleen and medulla of LNs (Fig. 1B). In contrast to secondary lymphoid organs (SLOs), E-cadherin$^+$ cells were observed at the portal vein of the liver and airways in the lung (Fig. 1B). However, naïve CD8 T cells and $T_M$ cells expressed neither E-cadherin nor N-cadherin in any of the organs tested (Fig. 1C, Supplementary Fig. S1) (Zhao et al., 2019). These results suggest that E- and N-cadherins are expressed on parenchymal cells in organs but not on naïve or $T_M$ cells.

### Cells expressing N-cadherin are co-localized with $T_M$ cells in the secondary lymphoid organs

Since E- and N-cadherins were expressed at locations where lymphocytes are known to be present, we hypothesized that these adhesion molecules are in contact with $T_M$ cells in organs. To test this hypothesis, we adoptively transferred the splenocytes obtained from P14 transgenic mice ($P14^+Ly5.1^+$), which contain CD8 T cells specific for LCMV, into naïve mice ($P14^-Ly5.2^+$). One day after the transfer, these mice were infected with LCMV-Armstrong (LCMV-Arm) to activate the P14 cells (Fig. 2A). At the memory phase (> day 30 post-infection, p.i.), the mice were euthanized, and immunofluorescence microscopy was performed to examine whether the adhesion molecules were co-localized with $T_M$ cells. The cells expressing N-cadherin in the spleen were co-localized with $T_M$ cells in the red pulp of the spleen (Fig. 2B), and approximately 75% of CD8 T cells highly expressing KLRG1 were in contact with N-cadherin (data not shown). However, N-cadherin did not appear to bind to the $T_M$ cells in the T-cell zone in the white pulp (Fig. 2B). Similar to the spleen, the cells expressing N-cadherin in the lymphatic vessels of LNs were also co-localized with $T_M$ cells (Fig. 2C, Supplementary Fig. S2). We also observed that E-cadherin$^+$ signals did not overlap with $T_M$ cells in the liver (Supplementary Fig. S3). These results that $T_M$ cells are co-localized with N-cadherin in the spleen and LNs, may indicate that $T_M$ cells receive signals in the form of adhesion molecules in SLOs.

### Blocking N-cadherin during the contraction phase reduces the number of $T_{CM}$-phenotype CD8$^+$ T cells in the spleen without impairing cytokine production

Since N-cadherin–expressing cells were co-localized with CD8 $T_M$ cells, we investigated if N-cadherin regulates the formation of $T_M$ cells. To do this, we treated LCMV-infected P14 chimeric mice with an N-cadherin–blocking mAb during the contraction phase (Fig. 3A). The results showed that, compared with the controls, the number of P14 cells in the animals treated with the N-cadherin-blocking mAb did not change in the SLOs or non-lymphoid tissues (NLTs) (Fig. 3B). Moreover, the numbers of cells highly expressing KLRG1 were comparable between N-cadherin–blocking mAb-treated mice and PBS–treated mice (Fig. 3C). However, N-cadherin in the spleen blockade decreased the number and slightly decreased frequency of $CD127^{hi}CD62L^{hi}$ $T_{CM}$ phenotype CD8 T cells in a dose-dependent fashion, while the number of $CD127^{hi}CD62L^{lo}$ cells was not affected (Fig. 3D, Supplementary Figs. S4 and S5).

To ascertain the role of N-cadherin in the acquisition of the secondary immune response by CD8 $T_M$ cells after re-infec-

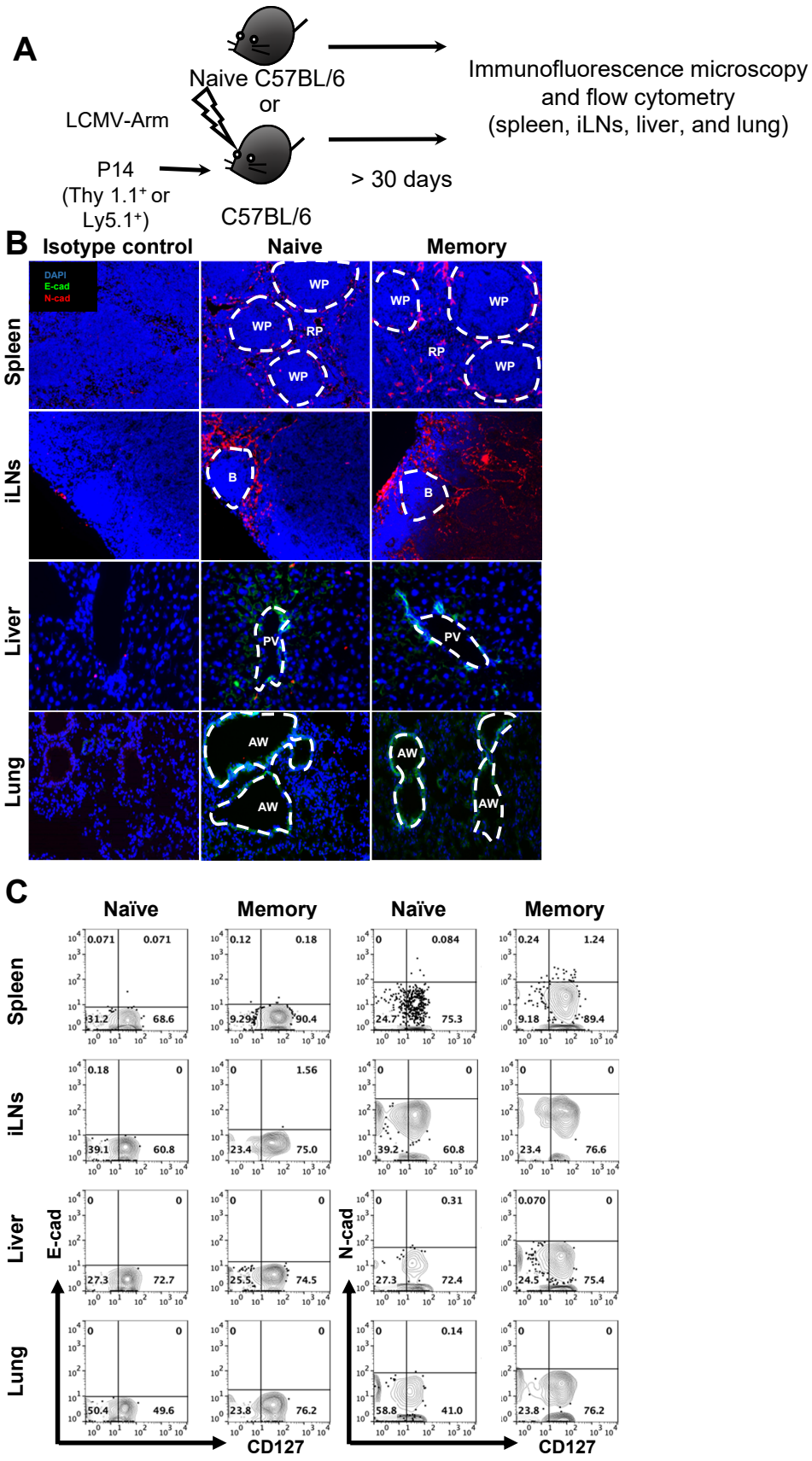


**Fig. 1. The expressions of E- and N-cadherin in the lymphoid and non-lymphoid organs.** An outline of the experimental design. (A) P14 (Thy 1.1$^+$ or Ly5.1$^+$); LCMV-specific Thy1.1$^+$ and Ly5.1$^+$CD8$^+$ T cells were used in (B) and (C), respectively. (B) Representative E- and N-cadherin expression in spleen, iLNs, liver, lung was observed in naïve B6 (referred as Naïve) or LCMV-infected memory mice (> day 30 p.i.) (referred as Memory) by using immunofluorescence microscopy. A rat IgG1 and non-biotinylated mAb were used as isotype control of an anti-E-cad or an anti-N-cad mAb, respectively. (C) Representative flow cytometric contour plots of E- and N-cadherin expression on naïve CD8$^+$ T cells from naïve B6 mice or Ly5.1$^+$CD8$^+$ T cells from LCMV-infected memory mice. RP, red pulp; WP, white pulp; B, B cell zone; PV, portal vein; AW, airway. The data shown are representatives of three independent experiments.

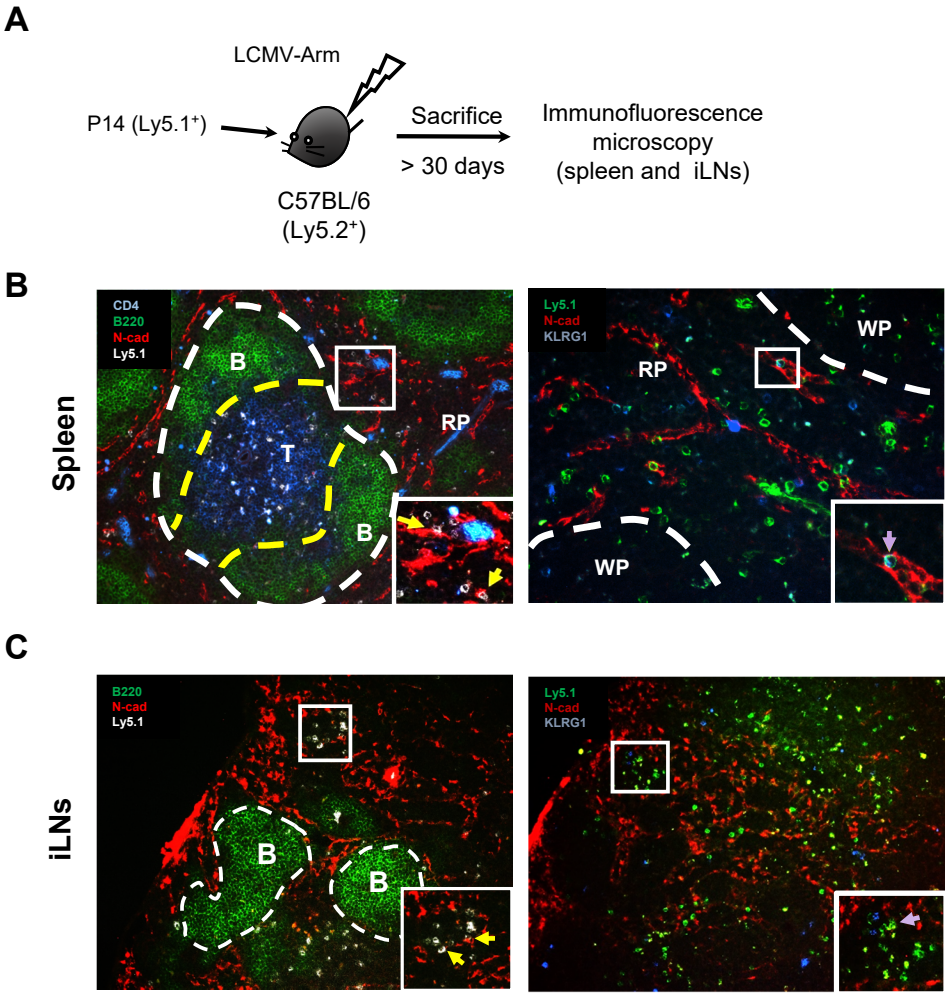


**Fig. 2. Co-localization of LCMV-specific CD8 $T_M$ cells with N-cadherin in the SLOs.** (A) Schematic illustration of the experimental design. The spleen (B) and iLNs (C) sections were fixed using acetone and then stained with indicated antibodies. The stained samples were analyzed by immunofluorescence microscopy. Yellow arrows and light purple arrows in each inlet indicated $P14^+$ $(Ly5.1)^+$ and $KLRG1^+$ $P14^+$ $(Ly5.1^+)$ $T_M$ cells that were co-localized with N-cadherin, respectively. B, B-cell zone; T, T-cell zone; RP, red pulp; WP, white pulp.

tion, we transplanted wild-type (WT) C57BL/6 mice with CD8 $T_M$ cells that had been developed without N-cadherin by the N-cadherin–blocking mAb treatment during the contraction phase. The recipient mice were infected with LCMV-Arm the day after the transfer of P14 $T_M$ cells (Fig. 4A). Compared with the control group, there was no further expansion of P14 cells and limited change in their TDEC and MPEC phenotypes in either SLOs or NLOs (Figs. 4B-4D, Supplementary Fig. S6). Furthermore, following viral peptide stimulation, pro-inflammatory cytokine production, including tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ), was not affected when N-cadherin was blocked during the contraction phase (Supplementary Fig. S7). Altogether, these results suggest that N-cadherin regulates the formation of CD8 $T_{CM}$ cells without impairing cytokine production.

### Blocking N-cadherin impaired the homing of $CD127^{lo}KLRG1^{hi}$ $T_M$ cells to the BM and inguinal lymph nodes

Since the production of CD8 T cells with the $T_{CM}$ phenotype declined after blocking N-cadherin in the spleen compared with the level in the control mice, we examined whether N-cadherin also modulates the homing of these cells to lymphoid organs. To answer this question, we adaptively transferred LCMV-specific memory CD8 T cells, isolated from the spleen of memory P14 chimeric mice, to WT naïve C57BL/6 mice and then administered the recipient mice with the anti–N-cadherin mAb every other day for 7 days (Fig. 5A). Despite treatment with an N-cadherin-blocking mAb, the numbers of P14 cells in SLOs and NLTs were comparable (Fig. 5B). However, unlike $CD127^{hi}KLRG1^{lo}$ cells, those with the $CD127^{lo}KLRG1^{hi}$ phenotype, known as the TDEC phenotype, were decreased in number in the BM of the recipient mice, and these cells were not even detectable in the inguinal lymph nodes (iLNs) (Fig. 5C, Supplementary Fig. S8). Moreover, although the number of cells with the $T_{CM}$ phenotype decreased after the antibody treatment during the contraction phase compared with the control level (Fig. 3D), these cells were not altered in the spleen of the memory P14 chimeric mice treated with the same antibody during the memory phase (Fig. 5D, Supplementary Fig. S8). Additionally, cytokine production in the $gp_{33-41}$-stimulated CD8 $T_M$ cells of memory P14 chimeric mice was not impaired by the treatment with anti–N-cadherin-blocking mAb compared with the level in PBS-treated mice (Supplementary Fig. S9). Taken together, these results show that N-cadherin influences the homing of terminally differentiated $CD127^{lo}KLRG1^{hi}$ $T_M$ cells to the BM and iLNs rather

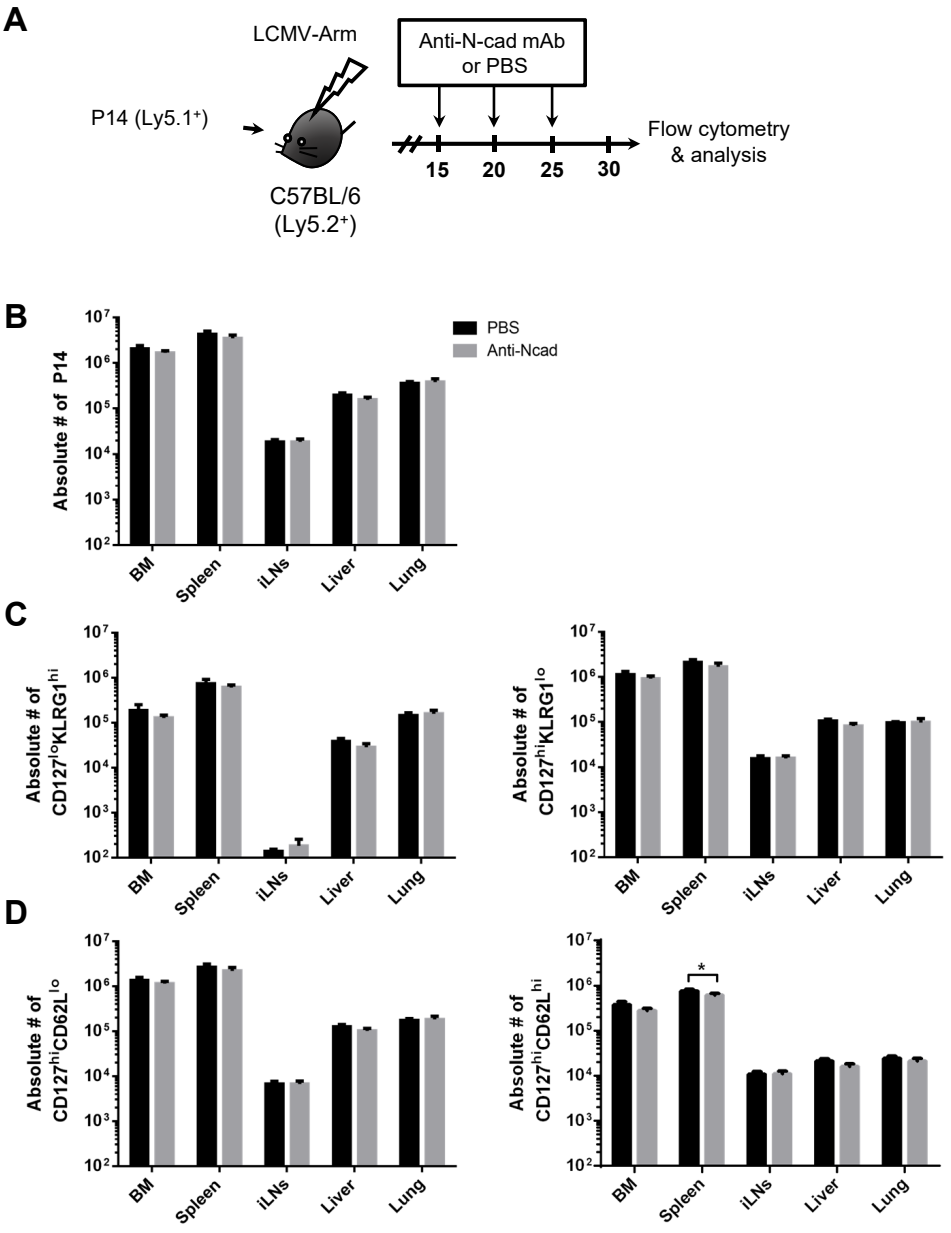


**Fig. 3. Decreased number of LCMV-specific CD127$^{hi}$CD62L$^{hi}$ CD8 $T_M$ cells in the spleen after the treatment of N-cadherin blockade during contraction phase.** (A) Schematic diagram of the experimental design. (B-D) The numbers of LCMV-specific Ly5.1$^+$CD8$^+$ $T_M$ cells (P14$^+$ (Ly5.1$^+$)) in the indicated organs were calculated based on flow cytometric analyses. Pooled data of n = 5 animals from two independent experiments. $*P < 0.05$. Error bars represent the mean ± SEM.

than regulating the functions of these cells.

## DISCUSSION

The development of long-term T cell-mediated immunity is crucial for protection against secondary infection as well in the design of new vaccines (Ahlers and Belyakov, 2010). The data presented here support the hypothesis that adhesion molecules provide important signals for the homeostasis of $T_M$ cells. N-cadherin was expressed in tissue cells, but not in naïve or $T_M$ cells. In addition, treatment with an N-cadherin–blocking mAb *in vivo* influenced the formation and maintenance of $T_M$ cells in lymphoid organs.

The expression of N-cadherin on HSCs and the importance of homophilic binding for their homeostasis have been reported (Alimperti and Andreadis, 2015). Although HSCs and $T_M$ cells showed similarities in microenvironment requirements, these data clearly suggest that the molecular mechanisms for the homeostasis of these cells differ. Contrary to our data showing that neither N-cadherin nor E-cadherin was expressed on the surfaces of naïve or $T_M$ cells, a $T_M$ cell population has been shown to express E-cadherin in the salivary gland, facilitating the recruitment of these $T_M$ cells (Hofmann and Pircher, 2011). Possible explanations for this observational discrepancy include 1) a different subset of $T_M$ cells was examined in the previous report, as the authors referred to a "unique" population of $T_M$ cells, and 2) the niches in the salivary gland differ from those in the SLOs in terms of the molecular mechanisms needed for the recruitment of $T_M$ cells. In agreement with our findings, a different report has shown

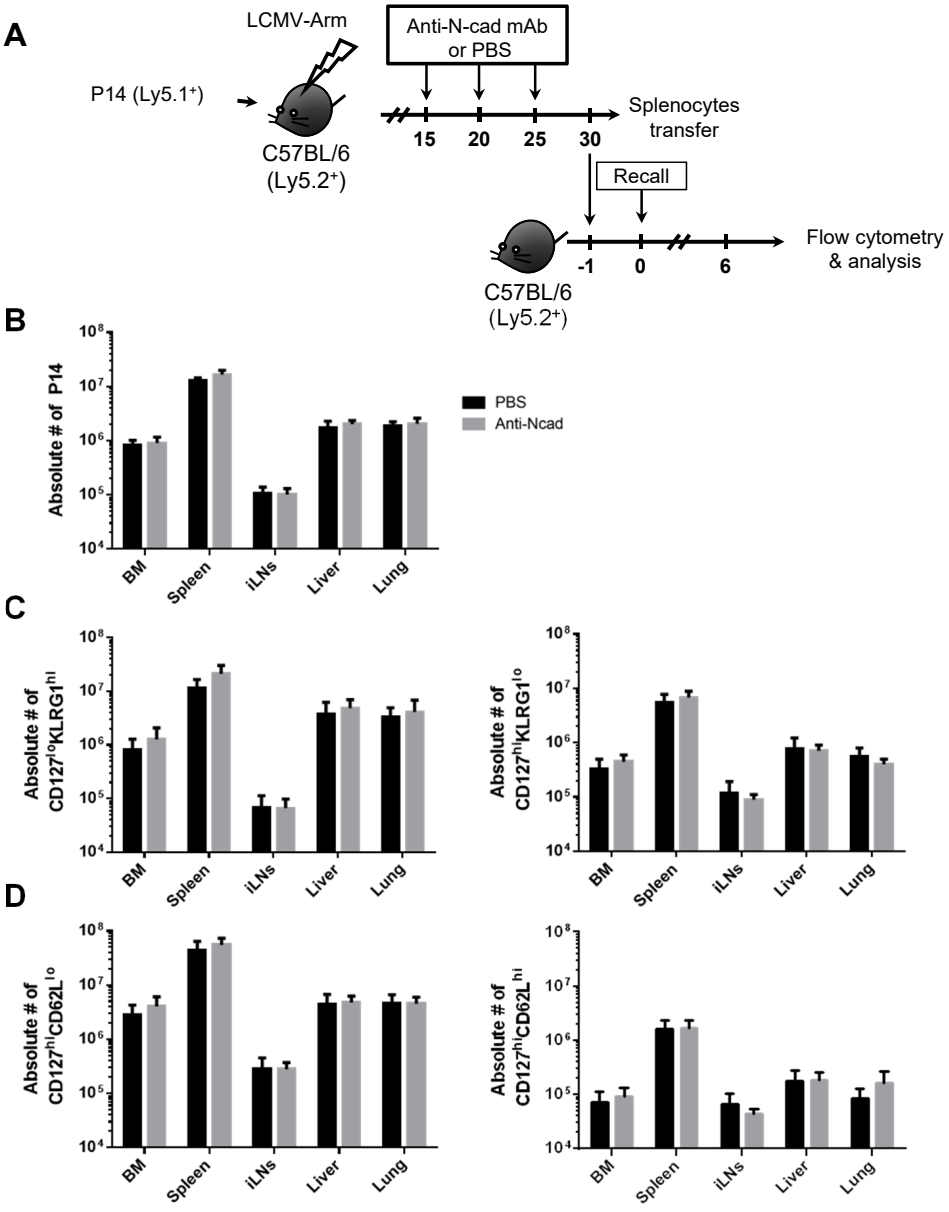


**Fig. 4. No effect of N-cadherin blockade during contraction phase for the expansion of memory P14 cells after the recall response.** (A) Schematic diagram of the experimental design. (B-D) The numbers of LCMV-specific Ly5.1$^+$CD8 $T_M$ cells in the indicated organs were calculated based on flow cytometric analyses. Pooled data of n = 5 animals from two independent experiments. Error bars represent the mean ± SEM.

no expression of E-cadherin on $T_{RM}$ cells. Instead, these cells bound to E-cadherin via their ligands, including CD103 (Topham and Reilly, 2018).

$T_{RM}$ cells that protect peripheral tissues without re-circulating are generally CD69$^{hi}$CD103$^{hi}$ (Topham and Reilly, 2018). These markers are important for their residency because CD69 downregulates sphingosine-1 phosphate receptor 1 (S1PR1), which mediates egress from tissues (Baeyens et al., 2015; Cyster and Schwab, 2012). In addition, CD103 has been proposed to anchor these cells by binding to E-cadherin, even though certain types of $T_{RM}$ cells do not express CD103 (Topham and Reilly, 2018). Since in this study we employed a systemic infection model in which circulating, not resident, $T_M$ cells generally develop, it was expected that E-cadherin blockade would have limited effect on the homeostasis of these $T_M$ cells.

N-cadherin, another type of cadherin, has been shown to form heterophilic binding with KLRG1, which is a well-defined marker for TDECs (Joshi et al., 2007). Upon binding to N-cadherin, KLRG1 recruits SHIP-1 and SHP-2 to inhibit the functions of NK and T cells (Tessmer et al., 2007). In addition, N-cadherin–expressing cardiac endothelial cells proliferate and induce angiogenesis by binding to KLRG1$^{hi}$ NK cells (Bouchentouf et al., 2010). These data suggest that signaling of KLRG1 and N-cadherin proceeds in both directions, leading to changes in the immune response as well as angiogenesis. Our finding that N-cadherin modulates the homeostasis of CD127$^{hi}$CD62L$^{hi}$ $T_M$ cells in the spleen adds another dimension to the roles of N-cadherin.

CD127$^{lo}$KLRG1$^{hi}$ $T_M$ cells did not properly home to the BM or LNs after adoptive transfer into naïve mice, followed by N-cadherin blockade. Since KLRG1 is a ligand for N-cadherin,

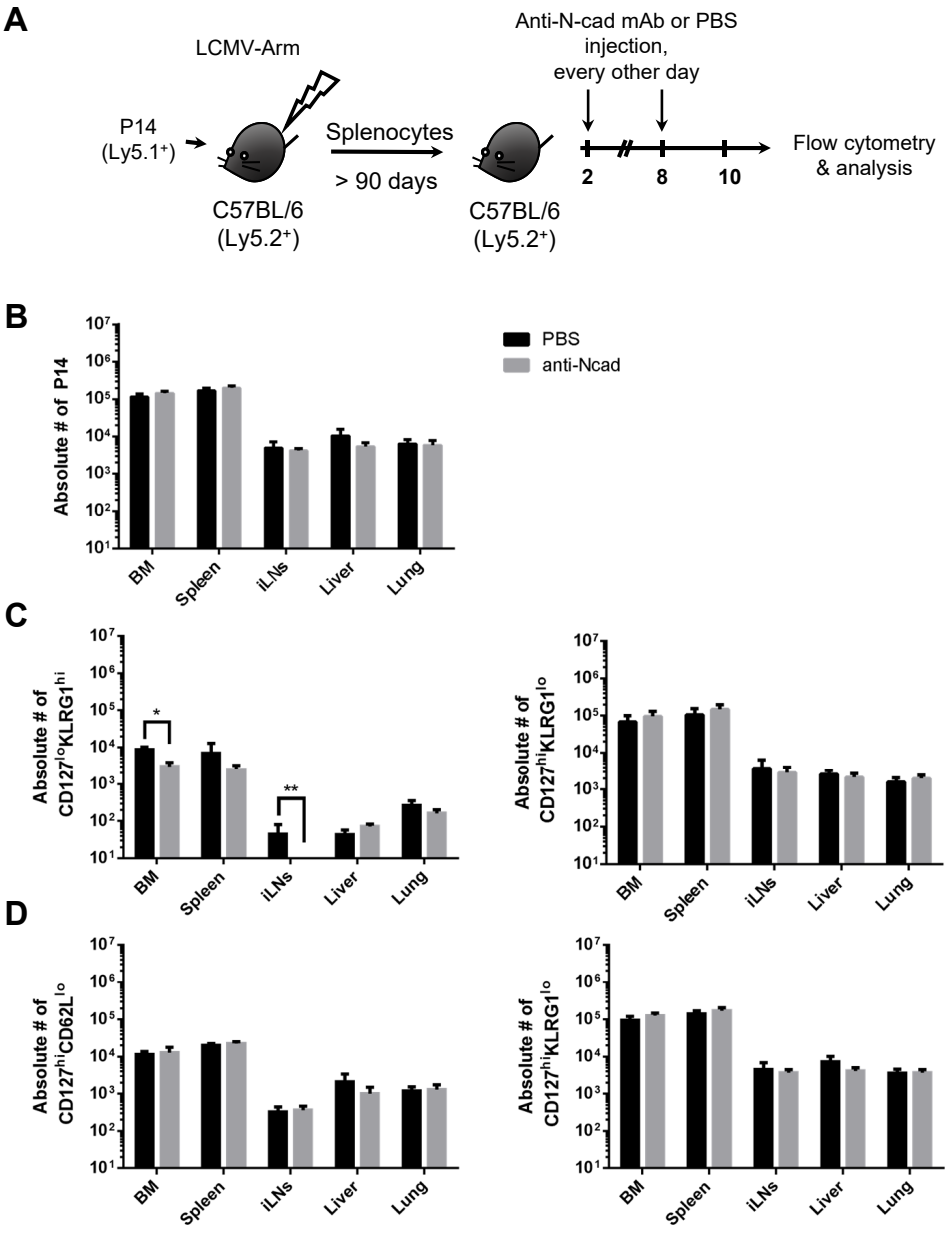


**Fig. 5. Declined LCMV-specific CD127$^{hi}$KLRG1$^{hi}$ CD8 $T_M$ cells in the BM and iLNs by blockade of N-cadherin during memory phase.** (A) Schematic diagram of the experimental design. (B-D) The numbers of LCMV-specific CD8 $T_M$ cells in the indicated organs were calculated based on flow cytometric analyses. (C) Pooled data of n = 5 animals from two independent experiments. Statistical analysis of the BM (Student's *t*-test) and iLNs (Wilcoxon signed-rank test). $*P < 0.05$; $**P < 0.01$. Error bars represent the mean ± SEM.

as mentioned above, and since N-cadherin was expressed in LNs, N-cadherin may have a role in recruiting KLRG1$^{hi}$ cells into SLOs. It would be interesting to examine N-cadherin expression in the BM and its co-localization with KLRG1$^{hi}$ $T_M$ cells. Of note, KLRG1$^{hi}$ $T_M$ cells in the spleen do not enter the parenchyma and instead circulates in the red pulp (Jung et al., 2010). Thus, the recruitment of this $T_M$ cell subset into the spleen was difficult to analyze through N-cadherin blockade.

This study revealed a novel role of N-cadherin in the homeostasis and recruitment of $T_M$ cells in SLOs. N-cadherin and E-cadherin were expressed in the SLOs and peripheral organs, respectively. We propose that N-cadherin plays a role in the recruitment of CD127$^{lo}$KLRG1$^{hi}$ $T_M$ cells into the BM and LNs, while this molecule helps to develop CD127$^{hi}$CD62L$^{hi}$ $T_M$ cells in the spleen during the contraction phase. Taken together, our findings provide additional evidence for the existence and importance of a niche for $T_M$ cell homeostasis.

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## AUTHOR CONTRIBUTIONS

K.H.K., A.C., and Y.W.J. conceived experiments. K.H.K., A.C., S.H.K., H.S., and Y.W.J. performed experiments. K.H.K., A.C., S.H.K., and Y.W.J. wrote the manuscript. S.J., K.K., J.J., and

H.C. provided expertise and feedback.

## CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

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