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CD62L Is Critical for Maturation and Accumulation of Murine Hepatic NK Cells in Response to Viral Infection

Hui Peng,* Rui Sun,*† Ling Tang,* Haiming Wei,*† and Zhigang Tian*†

NK cells play critical roles in the first line of defense against viruses and other pathogens. However, the factors that control NK cell recruitment into local sites to exert effector functions during viral infection remain poorly understood. In this study, we found that murine NK cells in various organs could be divided into CD62L− and CD62L+ subsets, the latter of which were less abundant in the liver and exhibited a relatively mature NK cell phenotype and a stronger cytotoxic function. Moreover, NK cells acquired CD62L expression after birth, and the frequency of CD62L+ NK cells gradually increased during postnatal development. In models of polyinosinic-polycytidylic acid administration and adenovirus infection in vivo, CD62L+ NK cell frequency and absolute numbers in the liver rapidly and markedly increased as a result of the augmented differentiation of CD62L− to CD62L+ NK cells and recruitment of peripheral mature NK cells to the liver. However, blocking CD62L prior to administering viral stimuli in vivo abolished viral stimulation–induced NK cell accumulation and maturation in the liver. Collectively, these data suggest that CD62L marks a mature NK cell subset, as well as affects the magnitude of the local NK cell response to viral infection. The Journal of Immunology, 2013, 190: 4255–4262.

Natural killer cells are key players in the innate immune system because of their high efficiency in cytotoxic function and cytokine production. As such, they are functionally important in several phases of the immune response: NK cells provide the first line of defense against pathogens, as well as contribute to shaping adaptive immune responses. To fulfill their effector functions, NK cells undergo a stepwise process of phenotypic and functional maturation. It is commonly believed that NK cells are predominantly generated from NK precursors (NKPs) in the bone marrow (BM), which express the β-chain of IL-2R (CD122) but lack NK cell surface receptors (1). NKPs then progressively acquire a series of surface-expressed molecules, such as acquisition of NK1.1 at an early stage and subsequent expression of Ly49 receptors and DX5 in mice (2, 3), to become fully competent for NK cell functions. Accumulating evidence demonstrates that NKPs and immature NK cells are present in mouse tissues other than the BM, including the liver, thymus, and lymph nodes (4–6), suggesting that an extra-BM pathway for NK cell development may exist.

The NK cell pool is composed of phenotypically heterogeneous subpopulations with distinct tissue distribution that are related to a broad range of adhesion molecules expressed by NK cells (7).

Under pathological conditions, such as infection or inflammation, NK cells can redistribute between organs and tissues and exert their effector functions locally at the pathological sites (7, 8). Several families of NK cell–expressed adhesion molecules are largely responsible for guiding NK cell homing and distribution, including selectins, chemokine receptors, and integrins. One member of the selectin family, CD62L (L-selectin), can initiate leukocyte tethering and rolling along the endothelium (9). Its expression on murine NK cells plays an important role in recruiting NK cells to draining lymph nodes (10) and in plasmacytoid dendritic cell–induced NK cell migration (11). Previous studies show that liver is enriched in NK cells that can quickly expand and accumulate in response to various stimuli (12, 13), including the innate immune stimulator polyinosinic-polycytidylic acid (poly I:C; synthetic dsRNA) (14) and viruses, such as adenovirus (15) or murine CMV (16), by a mechanism that is not fully understood. Whether CD62L plays a role in this NK cell expansion or accumulation in the liver has never been elucidated.

In addition to directing leukocyte migration, recent studies suggest that CD62L is differentially expressed during various stages of human NK cell maturation (17). High CD62L expression on CD56bright NK cells and heterogeneous CD62L expression on CD56dim NK cells separate human NK cells into three subsets that correlate with the sequential steps of NK cell maturation: CD56bright, CD56dim CD62L+, and CD56dim CD62L− (17). In mice, CD62L is expressed by the majority of peripheral NK cells (10) and at higher levels on CD11b− NK cells than on CD11b+ NK cells (18), suggestive of a correlation between CD62L expression and murine NK cell development.

In this study, we show that murine NK cells can be divided into two subsets depending on CD62L expression. Comparing the CD62L− and CD62L+ subsets in terms of their phenotype, function, and tissue distribution, CD62L+ NK cells represented the more mature subset. In response to poly I:C administration or adenovirus infection, CD62L+ NK cell frequency and numbers dramatically increased in the liver as the result of augmented differentiation from CD62L− NK cells, as well as enhanced NK cell recruitment from the periphery. Furthermore, CD62L blockade in vivo inhibited such stimuli-induced NK cell accumulation.

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Abbreviations used in this article: B6, C57BL/6; BM, bone marrow; CD11b+CD24−; CD11b+CD24−; CD11b+CD24−; CD11b+CD24−; DN; CD11b+CD24−; iLN, inguinal lymph node; KLRG1, killer cell lectin-like receptor G1; MNC, mononuclear cell; NK, NK precursor; poly IC, polyinosinic-polycytidylic acid.

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and maturation in the liver. Collectively, these results indicate that CD62L is required for NK cell maturation and accumulation in the liver under pathological conditions.

Materials and Methods

Mice

C57BL/6 (B6) mice were purchased from the Shanghai Experimental Animal Center (Shanghai, China). Rag1−/− (B6 background) mice were obtained from the Model Animal Research Center (Nanjing, China). CD45.1+B6.SJL mice were purchased from The Jackson Laboratory (Bar Harbor, ME). All mice were maintained under specific pathogen–free conditions and received care in accordance with guidelines outlined in the Guide for the Care and Use of Laboratory Animals.

Liver mononuclear cells (MNCs) and splenocytes were isolated as previously described (19). BM cells were obtained by flushing femur bones and then lysing erythrocytes. Peripheral blood MNCs were obtained after lysing erythrocytes. Single-cell suspensions of inguinal lymph node (iLN) cells were obtained by mechanical disruption.

Abs, flow cytometry, and cell sorting

Abs against CD3e (145-2C11), CD11b (M1/70), CD19 (ID3), CD27 (LG.3A10), CD43 (S7), CD45.2 (B6.D2), CD169 (H1.2F3), CD107a (1D4B), Ly49 C/I (5E6), NK1.1 (PK136), and IFN-γ (XMG1.2) were purchased from BD Biosciences. Abs against granzyme B (16G6), killer cell lectin-like receptor G1 (KLRG1; 2F1), and perforin (eBioOMAK-D) were purchased from eBioscience. Freshly isolated cells were incubated with rat serum to block nonspecific FcR binding and then stained with the indicated fluorescence-labeled mAbs for surface Ags. For CD107a staining, liver MNCs or splenocytes were incubated with YAC-1 cells at a 10:1 ratio with monensin (10 μg/ml Sigma-Aldrich) and PE anti-CD107a for 4 h before staining with other Abs. For intracellular cytokine staining, liver MNCs or splenocytes were stimulated with human rIL-2 (1000 U/ml; Kingsley) and murine rIL-12 (10 ng/ml; PeproTech) for 6 h, and monensin was added (10 μg/ml) during the last 4 h. After staining surface markers, cells were fixed, permeabilized, and stained with PE anti–IFN-γ, PE anti–granzyme B, and allophycocyanin anti-perforin. Stained cells were processed on an LSR II cytometer (BD Biosciences), and data were analyzed using FlowJo software (TreeStar).

Poly I:C injection and adenovirus infection

Poly I:C (Sigma-Aldrich) was dissolved in pathogen-free saline. Mice were injected i.p. with poly I:C at a dose of 30 mg/g body weight and sacrificed at 18 h after challenge. For adenovirus infection, type 5 adenovirus with deletions in the E1 and E3 regions (Beijing FivePlus Molecular Medicine Institute) was used. Mice were infected with 4 × 10¹⁰ adenovirus particles by i.v. injection and sacrificed at different time points. To evaluate the roles of CD62L in these two in vivo models, some mice were injected i.v. with 100 μg azide-free and low-endotoxin anti-CD62L (MEL-14; BD Biosciences) or isotype control (R35-95; BD Biosciences) mAbs 1 d before poly I:C injection or adenovirus infection.

Adoptive transfer

After splenocyte isolation from CD45.1+ B6 mice, CD62L− or CD62L+ NK cells were sorted using a FACS Aria Cell Sorter (BD Biosciences). Purified splenic NK cell subsets were then adoptively transferred into sublethally irradiated (600 rad given 1 d before adoptive transfer) CD45.2+ congenic mice, and recipients were sacrificed 12 d later. For the experiment evaluating the response of NK cell subsets to poly I:C treatment, hepatic CD62L− or CD62L+ NK cells from Rag1−/− (CD45.2+) mice were sorted using a FACS Aria Cell Sorter and adoptively transferred into nonirradiated CD45.1+ congenic mice that were treated with poly I:C 2 h later and sacrificed 20 h posttransfer.

Statistical analysis

Student t tests were used to determine statistically significant differences. The p values <0.05 were considered statistically significant.

Results

CD62L+ NK cells are abundant in the liver at steady state

The NK cell pool consists of a heterogeneous population of NK cells that exhibit divergent phenotypic and functional features, and different tissues contain NK cells at distinct stages of maturation (20). CD27 and CD11b expression was proposed to divide NK cells into four maturation stages: CD11b−CD27− (DN) → CD11blowCD27− (DP) → CD11b−CD27high (CD27low) (21). To determine the relative frequency of NK cells at different maturation stages within different tissues, we examined the expression of these surface molecules on NK cells in various mouse organs and tissues, including liver, spleen, peripheral blood, and iLN. Both the liver and BM were relatively enriched in immature DN and CD11blow NK cell subsets, which were far less frequent in the other examined tissues (Fig. 1). We also simultaneously tested CD62L expression on NK cells within these same tissues; interestingly, the frequency of CD62L+ NK cells was much lower in the liver and BM than in the spleen, blood, and iLN, which all contained predominantly CD62L− NK cells. Given that BM is the main site for NK cell development, and the liver is enriched in phenotypically immature NK cells (20), we produced a picture of the distribution of NK cell subsets in various tissues.
reasoned that CD62L+ NK cells may represent a more mature NK cell subset than CD62L− NK cells.

**CD62L+ NK cells exhibit a relatively mature phenotype**

To explore whether CD62L+ NK cells are more mature than CD62L− NK cells, we analyzed CD62L expression on NK cells at discrete developmental stages and compared the phenotype of other maturation-associated surface markers between the two subsets. Based on the four-stage scenario defined by CD11b and CD27 expression (21), we found that DN NK cells, the most immature, had the lowest CD62L+ cell frequency. This frequency increased upon maturation, with intermediate frequency among CD11blow NK cells and high frequency (mostly CD62L+) among the relatively mature CD11bhighCD27high and CD27low NK cells (Fig. 2A, 2B).

This tendency toward gradually increased CD62L expression levels during NK cell maturation was observed in liver, spleen, and BM, suggesting that this is a common phenomenon among NK cells, irrespective of their tissue origin.

Comparing the surface phenotype of CD62L+ and CD62L− NK cells, we found that the CD62L+ subset expressed lower levels of the CD51 integrin relative to the CD62L− subset but higher levels of NK cell maturation–associated markers, such as DX5, CD43, KLRG1, and Ly49 receptors (Fig. 2C). The expression levels of these surface markers on CD62L+ NK cells varied in different organs, further supporting the notion of NK cell diversity within different tissues. Nevertheless, these data collectively suggest that CD62L is a marker identifying NK cell subsets that exhibit a relatively mature phenotype.

**FIGURE 2.** Comparison of surface phenotype between CD62L+ and CD62L− NK cell subsets. (A) CD62L expression (solid lines) was determined on the indicated NK cell (NK1.1+CD3−CD19−) subsets in adult mouse liver by flow cytometry. Shaded curves represent isotype control staining. Data are representative of at least three independent experiments. (B) Percentages of cells expressing CD62L among the indicated NK cell (NK1.1+CD3−CD19−) subsets from liver, spleen, and BM of adult B6 mice (n = 5). Mean ± SEM are depicted. (C) The expression of the indicated surface markers was determined on the CD62L− NK cells (CD62L−NK1.1+CD3−CD19−; dashed lines) and CD62L+ NK cells (CD62L+NK1.1+CD3−CD19−; solid lines) by flow cytometry. Shaded curves represent isotype control staining. The statistical percentages are shown (n = 3–5) and expressed as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, paired Student t test.
CD62L\(^+\) and CD62L\(^-\) NK cell subsets differ in functional capacity

We next analyzed and compared the cytokine-producing and cytotoxic effector functions of CD62L\(^+\) and CD62L\(^-\) NK cell subsets to determine whether any functional differences exist between the subsets. IFN-\(\gamma\) is one of the prominent cytokines produced by NK cells. Splenic CD62L\(^+\) NK cells produced higher levels of IFN-\(\gamma\) than did CD62L\(^-\) NK cells in response to IL-2 and IL-12 stimulation (Fig. 3A); conversely, hepatic CD62L\(^+\) NK cells were less effective in producing IFN-\(\gamma\) than were their CD62L\(^-\) counterparts. Because cytotoxic activity is another important function for NK cells, we measured intracellular levels of granzyme B and perforin in hepatic and splenic CD62L\(^+\) and CD62L\(^-\) NK cell subsets (Fig. 3B, 3C). Hepatic CD62L\(^+\) NK cells expressed significantly higher levels of granzyme B and perforin compared with CD62L\(^-\) NK cells, indicating that hepatic CD62L\(^+\) NK cells possess more potent cytotoxic function; similar results were obtained in splenic CD62L\(^+\) NK cells for granzyme B. To further confirm these results, we examined the degranulation capacity of CD62L\(^-\) and CD62L\(^+\) NK cell subsets by measuring CD107a expression after incubation with YAC-1 target cells. Similar to previously reported results (22), we found that, overall, hepatic NK cells expressed higher levels of surface CD107a than did splenic NK cells, suggesting that hepatic NK cells have a stronger cytotoxic capacity than splenic NK cells. Moreover, CD62L\(^+\) NK cells expressed higher levels of CD107a than did CD62L\(^-\) NK cells; this was observed in both hepatic and splenic NK cell subsets (Fig. 3D), further indicating that CD62L\(^+\) NK cells exhibit higher cytotoxic activity than CD62L\(^-\) NK cells.

CD62L is gradually acquired and increases during postnatal NK cell development

Because CD62L\(^+\) NK cells are relatively mature in phenotype and function, we next wondered whether these NK cells appeared at a later stage of life compared with the CD62L\(^-\) subset. Thus, we examined the kinetics of CD62L expression on NK cells in mice at different ages. As expected, NK cells from newborns seldom expressed CD62L, although a large proportion of them expressed CD11b (Fig. 4A, 4B). At 1 wk of age, a small proportion of CD62L\(^+\) NK cells appeared in the liver, and approximately one third of splenic NK cells were CD62L\(^+\). CD62L\(^+\) NK cell frequency continued to increase gradually with age. We observed that splenic CD62L\(^+\) NK cells accumulated more rapidly and dramatically than did hepatic CD62L\(^+\) NK cells, possibly as a result of the unique liver environment that maintains immature NK cells. In adult mice, ~80% of splenic NK cells and 40% of hepatic NK cells were CD62L\(^+\), and the majority of these CD62L\(^+\) NK cells were CD11b\(^{high}\), which contrasts with the heterogeneous expression of CD11b on CD62L\(^+\) NK cells from young mice. These data indicate that NK cells gradually acquire CD62L expression during postnatal development and that this process is relatively suppressed in the hepatic environment.

We next sought to address whether the CD62L\(^+\) subset was generated from the CD62L\(^-\) subset in vivo. Thus, we adoptively transferred purified CD62L\(^-\) or CD62L\(^+\) NK cells from the spleen of CD45.1\(^+\) B6 mice into sublethally irradiated CD45.2\(^+\) congenic mice (Fig. 4C). Twelve days after transfer, we observed that a proportion of donor CD62L\(^-\) NK cells gave rise to CD62L\(^+\) NK cells in the recipient liver and spleen. However, transferred CD62L\(^-\) NK cells remained CD62L\(^-\) and did not differentiate into CD62L\(^+\) NK cells, suggesting that the conversion of CD62L\(^-\) to CD62L\(^+\) NK cells is irreversible.

Poly I:C promotes NK cell differentiation from CD62L\(^-\) to CD62L\(^+\) in vivo

It was reported that poly I:C, a synthetic viral RNA mimic, induces changes in NK cell phenotype (23). To evaluate whether viral stimulation alters CD62L expression on NK cells, we treated mice with poly I:C to mimic RNA virus infection. Consistent with previous reports (14, 23), the frequency and absolute number of total NK cells dramatically increased in the liver after poly I:C treatment (Fig. 5A, 5B). Notably, poly I:C induced significant upregulation of CD62L expression on hepatic NK cells (Fig. 5C), and the absolute number of CD62L\(^+\) NK cells also increased markedly (Fig. 5D). In contrast, the absolute number of CD62L\(^-\) NK cells did not change. This observed increase in hepatic CD62L\(^+\) NK cells could have been due to either the induction of CD62L expression on immature NK cells or the acquisition of CD62L during differentiation of mature NK cells from immature NK cells. Distinguishing between these possibilities, we found that expression of other maturation-associated markers, including CD11b and DX5, was also dramatically upregulated on hepatic NK cells (data not shown). Taken together, our data suggest that NK cells in the liver display a more mature phenotype after poly I:C stimulation and that CD62L is upregulated during this induced maturation.
To confirm that hepatic CD62L<sup>−</sup> NK cells rapidly differentiate into CD62L<sup>+</sup> NK cells upon poly I:C stimulation, purified hepatic CD62L<sup>−</sup> NK cells from Rag-1<sup>−/−</sup> (CD45.2+) mice were adoptively transferred into nonirradiated CD45.1+ congenic mice, and the recipients were injected with poly I:C 2 h later. Although a few CD45.2<sup>+</sup> cells differentiated into CD62L<sup>+</sup> B6 mice and adoptively transferred into sublethally irradiated CD45.2<sup>+</sup> congenic B6 mice. CD62L expression of donor NK cells (NK1.1<sup>+</sup>CD3<sup>−</sup>CD19<sup>−</sup>) before transfer is shown pre- and postsort. On day 12, CD62L expression was determined on hepatic and splenic NK cells of recipient mice by flow cytometry. Numbers in dot plots indicate percentages. Data are representative of two independent experiments.

FIGURE 4. NK cells gradually acquire CD62L expression during postnatal NK cell development. (A) CD62L and CD11b expression was determined on NK cells (NK1.1<sup>+</sup>CD3<sup>−</sup>CD19<sup>−</sup>) from liver and spleen of B6 mice at various ages by flow cytometry. Numbers in quadrants indicate percentages. Data are one representative of at least four individual mice. (B) Percentages of CD62L<sup>+</sup> NK cells among total NK cells at different ages (n = 4–6). Mean ± SEM are depicted. (C) A total of 1.5 x 10<sup>5</sup> CD62L<sup>−</sup> NK cells (CD62L<sup>−</sup>NK1.1<sup>+</sup>CD3<sup>−</sup>CD19<sup>−</sup>) or CD62L<sup>−</sup> NK cells (CD62L<sup>−</sup>NK1.1<sup>+</sup>CD3<sup>−</sup>CD19<sup>−</sup>) were isolated from the spleen of CD45.1<sup>+</sup> B6 mice and adoptively transferred into sublethally irradiated CD45.2<sup>+</sup> congenic B6 mice. CD62L expression of donor NK cells (NK1.1<sup>+</sup>CD3<sup>−</sup>CD19<sup>−</sup>) before transfer is shown pre- and postsort. On day 12, CD62L expression was determined on hepatic and splenic NK cells of recipient mice by flow cytometry. Numbers in dot plots indicate percentages. Data are representative of two independent experiments.

To confirm that hepatic CD62L<sup>−</sup> NK cells rapidly differentiate into CD62L<sup>+</sup> NK cells upon poly I:C stimulation, purified hepatic CD62L<sup>−</sup> NK cells from Rag-1<sup>−/−</sup> (CD45.2<sup>+</sup>) mice were adoptively transferred into nonirradiated CD45.1<sup>−</sup> congenic mice, and the recipients were injected with poly I:C 2 h later. Although a few CD45.2<sup>+</sup> cells differentiated into CD62L<sup>+</sup> NK cells 20 h after transfer in the livers of control recipients without poly I:C stimulation, the majority of CD45.2<sup>+</sup> cells in poly I:C–stimulated recipients were CD62L<sup>+</sup> (Fig. 5E), further confirming that poly I:C induced NK cells to acquire CD62L expression. Additionally, donor NK cell frequency in poly I:C–stimulated recipients was much higher than that in control recipients, suggesting that poly I:C treatment promotes NK cell homing to the liver.

**CD62L is required for poly I:C–induced NK cell accumulation and maturation in the liver**

To investigate whether CD62L is essential for NK cell accumulation and maturation in the liver after poly I:C stimulation in vivo, we treated mice with blocking anti-CD62L mAbs prior to poly I:C injection. Compared with control mAb–treated mice, anti-CD62L mAb–treated mice exhibited reduced NK cell frequency in the liver (Fig. 6A). Concomitantly, the absolute number of hepatic NK cells also decreased significantly (Fig. 6B). Although CD62L blockade at steady state also resulted in a trend toward decreased NK cell frequency and absolute numbers (Supplemental Fig. 1A, 1B), the downtrend was much less obvious than that observed between groups stimulated by poly I:C. Although NK cell phenotype did not change in normal mice not treated with poly I:C after blocking CD62L in vivo (Supplemental Fig. 1C), hepatic NK cells from poly I:C–stimulated mice that were pretreated with anti-CD62L mAbs showed higher frequencies of DN and CD11b<sup>low</sup> immature NK cells than did those from poly I:C–stimulated mice pretreated with control mAbs (Fig. 6D), exhibiting a phenotype closely similar to that of hepatic NK cells at steady state. In line with the results observed in the liver, NK cell frequency and numbers in the spleen and blood from poly I:C–stimulated mice also decreased in the anti-CD62L mAb–treated group compared with the control group (Supplemental Fig. 1D, 1E), suggesting that blocking CD62L does not induce NK cell migration from liver to the spleen and blood. Additionally, CD62L blockade did not inhibit poly I:C–induced NK cell activation, because CD69 expression on NK cells from anti-CD62L mAb–treated mice was extremely high and similar to that of control mAb–treated mice (Fig. 6C). Therefore, the data establish that CD62L is required for poly I:C–induced NK cell accumulation and maturation in the liver.
CD62L blockade impairs the hepatic NK cell response to adenovirus infection

To more broadly assess the role of CD62L in NK cell responses to viral infection, mice were infected with adenovirus, a non-enveloped dsDNA virus. NK cells were reported to mediate virus-induced liver injury and be required for priming T cell–mediated immunity in this model (24). In this study, we found that NK cell frequency in the liver increased markedly after systemic adenovirus infection that peaked at day 3 (Fig. 7A). Similar to poly I:C–treated mice, adenovirus-infected mice exhibited higher CD62L expression on hepatic NK cells compared with uninfected mice (Fig. 7B). CD62L is required for NK cell accumulation in the liver during adenovirus infection, because the adenovirus-induced increase in hepatic NK cells was abolished in mice pretreated with anti-CD62L mAb (Fig. 7C). Splenic NK cell frequency and numbers also decreased in the anti-CD62L group compared with the control group (Supplemental Fig. 1F, 1G). Furthermore, CD62L blockade maintained the relative abundance of immature NK cells found in the livers of uninfected mice, which was reflected in the increased proportion of CD11blow NK cells in mice pretreated with anti-CD62L (Fig. 7D). Thus, blocking CD62L inhibits NK cell accumulation in the liver and restrains their maturation, which may reduce the efficiency of NK cell responses to viral infection and affect the induction of adaptive immunity.

Discussion

NK cell maturation is a multistage process that has long been an area of intense research interest. Although it is widely accepted that the BM is the primary site for NK cell development, recent evidence suggests that NK cells can also undergo further differentiation at peripheral sites, such as licensing (25), and the acquisition of KLRG1 (26) outside the BM. In this study, we observed heterogeneous CD62L expression on murine NK cells and demonstrated that CD62L+ NK cells, which are less abundant in the liver and BM compared with other organs, exhibit a mature phenotype and stronger cytotoxic activity compared with the CD62L− subset. In addition, we found that NK cells gradually acquire CD62L expression during postnatal development and that CD62L− NK cells (CD62L−NK1.1+CD3−CD19−) was isolated from the liver of Rag-1−/− (CD45.2−) mice and adoptively transferred into nonirradiated CD45.1+ congenic B6 mice, which were then treated with poly I:C 2 h posttransfer and sacrificed 18 h after poly I:C treatment. CD62L expression was determined on hepatic NK cells (NK1.1+CD3−CD19−) of recipient mice by flow cytometry. Numbers in quadrants indicate percentages. The percentages of CD45.2+CD62L+ NK cells among CD45.2+ NK cells of recipient mice are shown (n = 3) and expressed as mean ± SEM. **p < 0.01, ***p < 0.0001, unpaired Student t test. ns, Not statistically significant.

FIGURE 5. The differentiation of CD62L− NK cells into CD62L+ is augmented by poly I:C stimulation. (A–D) Liver leukocytes were isolated from B6 mice at 18 h postinjection of poly I:C or PBS and analyzed by flow cytometry. (A) Percentages of hepatic NK cells (NK1.1+CD3−CD19−) from poly I:C- or PBS-treated mice. Data are representative of three independent experiments. (B) Absolute hepatic NK cell numbers (n = 5) expressed as mean ± SEM. **p < 0.005, unpaired Student t test. (C) CD62L expression (solid lines) on hepatic NK cells (NK1.1+CD3−CD19−). Shaded curves represent isotype control staining, and numbers indicate percentages. Data are representative of three independent experiments. (D) Absolute cell numbers of CD62L− NK cells (CD62L−NK1.1+CD3−CD19−) and CD62L+ NK cells (CD62L+NK1.1+CD3−CD19−) from liver of poly I:C-treated and control mice (n = 5) and expressed as mean ± SEM. **p < 0.005, unpaired Student t test. (E) A total of 105 CD62L− NK cells (CD62L−NK1.1+) or CD62L+ NK cells (CD62L−NK1.1−) was isolated from the liver of poly I:C-treated and control mice (n = 5) and expressed as mean ± SEM. **p < 0.01, ***p < 0.0001, unpaired Student t test. ns, Not statistically significant.
As important effectors of the innate immune system, NK cells play a significant role in host defense against viral infection and tumor growth. NK cells are sensitive to various pathogenic stimuli and are activated quickly by recognizing stimuli in a way that does not require previous sensitization to eliminate pathogens (27). NK cell antiviral function is mediated by multiple mechanisms, including direct cytotoxicity, Ab-dependent cell-mediated cytotoxicity, Th1-type cytokine production, and cross-talk with dendritic cells (28). Based on these mechanisms, NK cells participate in innate immunity, as well as in shaping the adaptive-immune response by a complex interaction network with other distinct cells.

In particular, the liver is selectively enriched in NK cells compared with other organs, and a considerable proportion of these hepatic NK cells display an immature phenotype (3, 4). Previous reports, as well as our present study, show that a variety of stimuli, including synthetic viral mimics, like poly I:C (14, 23), or natural virus infection, like adenovirus (15, 24), rapidly induce NK cell activation and accumulation in the liver, which provides a useful window to study the NK cell–driven antiviral response. Moreover, as shown in the current study, hepatic NK cells exhibited a relatively mature phenotype following poly I:C treatment or adenovirus infection, with a lower frequency of immature DN and CD11b^low^ NK cells than those from untreated control mice. In both models, CD62L expression on hepatic NK cells increased markedly, further confirming that CD62L defines a relatively mature NK cell subset. Our adoptive-transfer experiments investigating the source of the increased mature NK cells in the liver demonstrated that differentiation of CD62L^+^ into CD62L^−^ NK cells and preferential NK cell recruitment to the liver were augmented upon poly I:C injection. Therefore, both the differentiation of local immature hepatic NK cells and the recruitment of peripheral NK cells into the liver contribute to the increased frequency and absolute number of mature NK cells in the liver.

As a member of the selectin family, CD62L is of great importance for leukocyte homing to peripheral lymph nodes and inflamed sites (29–31), leading us to speculate that CD62L might also be required for NK cell accumulation in the liver following pathogenic stimulation. Indeed, we observed that blocking CD62L significantly reduced NK cells in the liver in both the poly I:C and adenovirus infection models. Given that poly I:C may induce recruitment of splenic NK cells into the liver (8), we expected that retention of NK cells in the spleen would likely be rescued in mice pretreated with anti-CD62L mAb. Surprisingly, we found that splenic NK cell numbers did not return to normal by blocking CD62L before poly I:C stimulation; in fact, the numbers were much lower than in control mice, which was the same result

![Figures 6 and 7](http://www.jimmunol.org/)

**FIGURE 6.** Poly I:C-induced NK cell accumulation and maturation in the liver are dependent on CD62L. B6 mice were injected with anti-CD62L or isotype control mAb 1 d before poly I:C treatment. The frequency (A) and absolute cell number (B) of hepatic NK cells (NK1.1^+^CD3^+^CD19^−^) were enumerated from each group of mice by flow cytometry. Numbers in flow plots indicate percentages. (C) CD69 expression (dashed lines for control group; solid lines for anti-CD62L group) on hepatic NK cells (NK1.1^+^CD3^+^CD19^−^) was determined by flow cytometry. Shaded curves represent isotype control staining. (D) CD11b and CD27 expression on hepatic NK cells (NK1.1^+^CD3^+^CD19^−^) was determined by flow cytometry. Numbers in quadrants indicate percentages. Data are mean ± SEM (n = 4). **p < 0.01, ***p < 0.0001, unpaired Student t test. ns, Not statistically significant.

**FIGURE 7.** Inhibition of CD62L compromises the hepatic NK cell response to adenovirus infection. (A) B6 mice were inoculated with 4 × 10^10^ adenovirus particles and killed at the indicated times. The frequency of hepatic NK cells (NK1.1^+^CD3^+^CD19^−^) was determined by flow cytometry (n = 4). Mean ± SEM are depicted. (B) Flow cytometric analysis of CD62L expression (solid lines) on hepatic NK cells (NK1.1^+^CD3^+^CD19^−^) of B6 mice 1 d after adenovirus infection. Shaded curves represent isotype control staining. Data are representative of two independent experiments. (C and D) B6 mice were treated with anti-CD62L or isotype control mAbs 1 d prior to adenovirus infection. The frequency of hepatic NK cells (NK1.1^+^CD3^+^CD19^−^) was enumerated at 1 d postinfection (C), and the expression of CD11b and CD27 (D) was determined by flow cytometry. Numbers in dot plots indicate percentages (n = 4). Data are mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, unpaired Student t test.
obtained in the adenovirus infection model. Thus, how CD62L impacts NK cell accumulation in the liver remains unanswered. Nevertheless, a new role for CD62L influencing NK cell accumulation has been revealed that has important implications for CD62L as a potential therapeutic target for hepatitis, because inhibition of CD62L reduces NK cell numbers in the liver and, therefore, may impair the magnitude of NK cell responses and the subsequent generation of virus-specific adaptive immunity.

In conclusion, the data presented in this study demonstrate that CD62L expression defines a relatively mature NK cell subset. Following viral infection, NK cells in the liver increase significantly and upregulate CD62L expression, concomitant with the acquisition of a more mature NK cell phenotype. CD62L is required for these changes, because they can be reversed by pretreatment with blocking anti-CD62L mAbs. Although the mechanisms underlying how CD62L affects NK cell responses, as well as the final downstream consequences of CD62L inhibition, remain to be investigated, we provide evidence that CD62L plays a critical role for NK cell accumulation at local sites of viral infection; this will shed more light on understanding the mechanisms of host response to virus-induced human diseases.

Disclosures
The authors have no financial conflicts of interest.

References