An Essential Role of Innate Lymphoid Cells in the Pathophysiology of Graft-vs.-Host Disease

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INTRODUCTION

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Keywords: innate lymphoid cells, graft-vs.-host disease, NK cells, T cells, hematopoietic stem cell transplantation, ILCreg

INTRODUCTION

Definition of ILCs

Innate lymphoid cells (ILCs) encompass natural killer cells (NK) and ILC1, ILC2, and ILC3 cells (1–3). In contrast to T cells, these cells lack rearranged antigen receptors (1–3). It has been demonstrated that ILCs develop in the fetal liver and adult bone marrow, whereas mature ILCs are mainly enriched in the GI tract, lungs, liver, and skin (1–3). NK cells, which account for ~15% of human peripheral blood (PB) lymphocytes, exert cytolytic effects, and secrete IFN-γ, granzyme B, and perforin. In mouse, NK cells are characterized by the expression of natural killer cell p46-related protein (NKp46; also known as NCR1) receptor, and expressing transcription factors T-bet and Eomes (4–6). Murine ILC1 cells express Nkp46, NK1.1, T-bet, and CD200r1, but without expression of Eomes (4–6). However, unlike NK cells, ILC1 cells do not display cytolytic effects (15). Murine ILC1 cells express Nkp46, NK1.1, T-bet, and CD200r1, but without expression of Eomes (16).

In humans, CD127+CD161+CD56dim T-cells express IFN-γ+ILC1 cells are enriched in the tonsils (15–17). Interestingly, Lin−CD127+CD161+CD117−NKP44−CRTH2− ILC1 cells have been found in the PBMCs of healthy individuals and atopic dermatitis (AD) patients (15–18).
Characteristics of ILCs. ILCs encompass NK, ILC1, ILC2, and ILC3 cells. Murine and human NK cells can secrete IFN-γ, granzyme B, and perforin. In humans, NK cells have two main subsets: CD56brightCD16− and CD56dimCD16+ cells. ILC1 cells can respond to IL-12 and IL-15, and subsequently produce IFN-γ and TNF-α. In humans, CD127+CD161+CD25+KLRG1+GATA3high ILC1 cells are enriched in the tonsils. Additionally, Lin−CD127+CD161+CD56+CRTH2+ILC1 cells have been found in the human PBMCs. In mice, ILC2 cells are Lin−CD127+CD25+KLRG1+GATA3high cells which are responsive to IL-2, IL-4, IL-7, IL-25, TSLP, and prostaglandin D2, and subsequently produce multiple effector cytokines. In humans, ILC2 cells express GATA3, CD127, CD161, CD25, ST-2, IL-17A, and CRTH2. Both murine and human ILC3 cells are Lin−CD127+RORγt+. They are responsive to IL-1β, IL-6, and IL-23, and produce IL-22, IL-17A, IL-17F, GM-CSF, TNF-α, and LTα1β2.
### TABLE 1 | Phenotype of murine ILCs.

| Marker | Mouse | ILC1 | ILC2 | ILC3 |
|--------|-------|------|------|------|
| CD3    | – (7) | – (7) | – (7) | – (7) |
| CD4    | – (8) | – (9) | – (9) | ± (7, 9) |
| CD19   | – (7) | – (7) | – (7) | – (7) |
| CD25   | ± (10) | ± (10) | + (7, 10) | ± (10) |
| CD45   | + (10) | + (7, 10) | + (7, 10) | + (7, 10, 11) |
| CD49a  | ± (7, 10) | + (7, 10) | ND | – ND |
| CD69   | ± (10) | + (10) | ND | – ND |
| CD90   | ± (10) | + (10) | + (10) | + (10) |
| CD94   | ± (10) | ND | – ± (10) | ND |
| CD103  | ± (10) | – (10) | ND | – ND |
| CD117  | – (10) | ± (10) | ± (10) | ± (10) |
| CD122  | + (10) | + (10) | + (10) | – (10) |
| CD127  | ± (10) | ± (9, 10) | + (9, 10) | + (7, 9, 10) |
| CD160  | ± (10) | + (10) | ND | – ND |
| CD294  | – (10) | ND | – | + (10) | ND |
| NKP48  | + (7, 10) | + (7, 10) | – (10) | ± (7, 10, 11) |
| NK1.1  | + (7, 10) | + (7, 10) | – (10) | ± (10) |
| NKG2D  | + (10) | ND | – | – (10) | ± (10) |

ND: not determined.  
+ positive; – negative; ± sometimes positive.

### TABLE 2 | Phenotype of human ILCs.

| Marker | Human | ILC1 | ILC2 | ILC3 |
|--------|-------|------|------|------|
| CD1a   | – (5, 12) | – (5, 12) | – (5, 12) | – (5, 12) |
| CD3    | – (12) | – (5, 12) | – (12) | – (12) |
| CD4    | – (13) | ± (14) | – (13) | ± (15) |
| CD7    | + ND | ± (9) | + (9) | + (9) |
| CD11c  | – (5, 12) | – (5, 12) | – (5, 12) | – (5, 12) |
| CD14   | – (5, 12) | – (5, 12) | – (5, 12) | – (5, 12) |
| CD16   | ± (10, 15) | – (10) | – (10) | – (10) |
| CD19   | – (5, 12) | – (5, 12) | – (5, 12) | – (5, 12) |
| CD25   | ± (10) | + (10) | + (10, 14, 15) | ± (10) |
| CD34   | – (5, 12) | – (5, 12) | – (5, 12) | – (5, 12) |
| CD45   | + (5, 10) | + (10) | + (10) | + (5, 10) |
| CD49a  | ± (15) | ± (15) | ND | – ND |
| CD66   | + (10, 15) | – (5, 10) | – (9, 10) | ± (9, 10) |
| CD69   | ± (10) | ± (10) | ND | – + (5) |
| CD84   | ± (5, 10) | – (5, 10, 12) | – (5, 10, 12) | – (5, 10, 12) |
| CD103  | ± (15) | ± (9, 15) | – (9) | – (9) |
| CD117  | ± (10) | – (10) | ± (10) | – (10) |
| CD123  | + (5, 12) | – (5, 12) | – (5, 12) | – (5, 12) |
| CD127  | ± (10) | ± (10) | + (5, 10, 13) | + (5, 10) |
| CD294  | – (12) | – (12) | + (5, 12) | – (12) |
| TCRγδ | – (12) | – (12) | – (12) | – (12) |
| NKP48  | + (10) | – (10) | – (10) | ± (10) |
| NKP44  | ± (10) | ± (10) | – (10) | ± (10) |
| NKP30  | ± (10) | ± (10) | ± (10) | ± (10) |
| NK1.1  | ± (10) | + (5, 10) | + (10) | ± (10) |
| NKG2D  | + (10) | ND | – ND | – (10) |

ND: not determined.  
+ positive; – negative; ± sometimes positive.

### Generation, Transcription, and Plasticity of ILCs

ILCs originate from common lymphoid progenitors (CLPs), which subsequently differentiate into two different lineages: the common helper-like innate lymphocyte progenitors (CHILPs) and the conventional natural killer cell progenitors (cNKps) (Figure 2). However, CHILPs are a heterogeneous population consisting of innate lymphoid cell precursors (ILCPs) and lymphoid tissue-inducer precursors (LTPs) (33, 34). CHILPs are defined as Lin<sup>−</sup>CD127<sup>+</sup>CD25<sup>+</sup>RORγ<sup>+</sup> cells (15). Mouse ILC3 cells consist of three subsets: lymphoid tissue-inducer cells (LTI), LTI-like CCR6-expressing ILC3 cells and NCR-expressing ILC3 cells (NCR<sup>+</sup>ILC3) (1, 15). Similar to Th17 cells, they are poised to respond to the stimulation by IL-1β, IL-6, and IL-23 and subsequently produce effector cytokines, such as IL-22, IL-17A, IL-17F, GM-CSF, TNF-α, and LTα1β2 (1, 15, 27–29).

NK cells are critical players in controlling intracellular bacterial and tumor surveillance (1, 15, 30). ILC1 cells are capable of controlling intracellular pathogens, whereas ILC2 cells have the capacity to limit extracellular parasitic worm infections, promote epithelial repair, and maintain mucosal tissue homeostasis. Notably, ILC2 cells are associated with chronic diseases such as pulmonary fibrosis, hepatic fibrosis, and atopic dermatitis (1, 2, 15, 30). NCR<sup>+</sup> ILC3 cells are the most prevalent ILC3 subset in the intestine, whereas LTI-like ILC3 cells are mainly localized in the colon and lymphoid tissues (2, 30–32). ILC3 cells are key contributors to tissue repair and protect mucosal barriers against infection by extracellular bacterial and fungi (1, 2, 30–32).
However, the functional maturation and bone marrow egress of these cells requires T-bet (45–48). NFIL3 is involved in the development of bone marrow-derived NK cells from CLPs under homeostatic conditions and is necessary for the formation of splenic and thymic NK cells (39–42). Unlike cNK cells, ILC1 cells arise from Id2⁺ PLZF⁺ CHILP progenitor cells (49). Interestingly, the development of ILC2 cells requires Id2 (36, 37), GATA-binding protein 3 (GATA-3) (50–52), RORa (53), transcription factor 1 (TF-1) (54–56), BCL11B (57, 58), and Notch (59, 60). GATA-3 is crucial for the secretion of effector cytokines, such as IL-5 and IL-13, by mature ILC2 cells (50–52, 61). In addition, Gfi1 can promote the development of ILC2 cells and control their responsiveness during infection by *Nippostrongylus brasiliensis* and protect against allergen-induced lung inflammation (62). Runx3 is another key factor in the differentiation of ILC1 and ILC3 cells. It controls the survival of ILC1 cells and is necessary for the expression of RORγt and AHR in ILC3 cells (7, 63).

ILC3 cells differentiate from Lin⁻IL-7Rα⁺Flt3⁻γc⁻⁻⁺ fetal liver progenitors and express Id2 and RORγt in mice (1, 37). The development of murine LTi cells and LTi-like ILC3 cells requires the expression of RORγt, the aryl hydrocarbon receptor (AHR), RUNX3 and Notch (1, 2, 37, 64). The AHR seems to be involved in the expansion of CCR6⁻/~ILC3 cells (65–68). AHR⁻/⁻ mice exhibit a decrease in CCR6⁻/~ILC3 cells without alteration in the CCR6⁺ILC3 population. Furthermore, T-bet controls the fate and function of CCR6⁺RORγt⁺ ILCs. Postnatal CCR6⁻RORγt⁺ ILCs upregulate T-bet, which is modulated by the commensal microbiota. Tbx21⁻/⁻ mice exhibit normal development of CCR6⁺RORγt⁺ cells but they fail to differentiate into NKp46⁺RORγt⁺ ILCs, suggesting that T-bet is necessary for the differentiation of NKp46⁺RORγt⁺ ILCs in mice (8, 69). Additionally, the IL-1β/IL-1R/MyD88 pathway controls the production of IL-22 by NKp46⁺RORγt⁺ ILCs in the small intestine (SI) of mice (70). In contrast to mice, both human Lin⁻CD34⁺CD45RA⁺CD117⁺IL-1R⁺RORγt⁺ and stage 2 IL-1R⁺ cells in secondary lymphoid tissues (SLT) can differentiate into nearly all ILC populations including NK cells (71). Collectively, these results demonstrate that the development of ILCs is not dependent on a single “master regulator” but on a complex network of transcription factors (TFs) (1, 15, 31). Interestingly, recent studies have focused on the plasticity of ILCs. For instance, ILC1 cells can be converted into NK cells after ectopic expression of Eomes (31, 48). IL-12 can endow ILC2 cells with ILC1 features by secreting IFN-γ (60, 72), whereas IL-12 and IL-23 can induce the transition of ILC3 cells into ILC1 cells (60, 73, 74). Furthermore, dermal NCR⁺ILC3 cells can be transformed into NCR⁺ILC3 cells in the presence of IL-18 and IL-23 in vitro (42, 75–77).

**Localization and Migration of ILCs**

NK cells are mainly located in the bone marrow, lymph nodes, spleen, lungs, and liver, whereas ILC1 cells mainly reside in the intestinal intraepithelia (IE) (2, 78, 79). ILC2 cells are located in the lungs and lamina propria of the small intestine (SI) and skin, whereas ILC3 cells are predominantly located in the lamina propria, Peyer’s patches and lymphoid follicles of the small intestine (78, 79). It is generally considered that fetal liver and bone marrow are the “factories” where ILC subsets are generated (1, 2). However, a report by Gasteiger et al. have indicated that the vast majority of ILCs in both lymphoid and non-lymphoid organs are long-lived tissue-resident under steady state (80). Another elegant study by Di Santo JP’s lab has proposed a model of “ILC-poiesis” and provided a mechanism by which tissue ILCs could be replenished from blood ILCPs in response to steady-state losses and under the circumstance of infection and inflammation (81–83).

Recently, increasing evidence has indicated that ILC1 and ILC3 cells can migrate into SLTs, depending on integrins and
chemo-attractant receptors, whereas the migration of ILC2 cells from hematopoietic sites to target tissues is independent of the aforementioned receptors.

It has been indicated that the migration of NK cells to LNs via high endothelial cells (HEVs) might be mediated by CCR7 or CXCR3. The migration of ILC1 and ILC3 cells to SLTs occurs in a CCR7-dependent manner (84, 85). ILC2 cells, located in the bone marrow, spleen as well as mesenteric lymph nodes, constitutively express CCR9 and α4β7, rather than the RA-dependent homing receptor (79, 84). The migration of LTi-ILC3 cells to lymphoid follicles and the spleen marginal zone is regulated by the CXCL13-CXCR5 axis (86). Notably, trafficking receptor switches play a crucial role in the migration of ILCs. For instance, activation of spleen ILC3 cells induces upregulation of CCR9 and α4β7 with concomitant downregulation of CCR7 in the presence of IL-7 and all-trans retinoic acid (RA) and prompts the migration of these cells to the intestine (84, 87, 88).

ILCs and GVHD

Allogeneic hematopoietic stem cell transplantation (Allo-HSCT) is the most powerful therapy for hematologic malignancies and a majority of non-malignant hematological diseases. One of the major barriers to the efficacy of allo-HSCT is the occurrence of GVHD. Radiotherapy/chemotherapy induction regimens damage epithelia, especially the intestinal mucosa, in recipients, followed by the translocation of commensal microflora from the GI tract into the peripheral blood. Subsequent activation of adaptive immunity promotes the occurrence of aGVHD (89–94).

The Role of Donor-Derived ILCs in GVHD

The role of NK cells in the pathogenesis of GVHD seems to be controversial (95, 96). Early studies indicated that target organs, such as the skin, liver, and GI tract, in HSCT recipients with aGVHD were infiltrated with NK cells, suggesting that NK cells might promote the development of GVHD (97–99). In accordance, administration of NK cell depleting antibodies against GM1 or NK1.1 significantly mitigated GVHD in murine models (100, 101). Cooley et al. have demonstrated that, in unrelated HSCT, increased production of IFN-γ by NK cells has correlated with more aGVHD, and decreased KIR expression has associated with inferior survival of patients, suggesting that NK cells might promote GVHD via secretion of inflammatory cytokines such as IFN-γ and TNF-α (102).

Recently, a first-in-human trial of adoptive transfer of donor-derived IL-15/4-1BBL-activated NK cells was conducted in an HLA-matched, T-cell-depleted non-myeloablative peripheral blood stem cell transplantation (103). In this clinical trial, five of nine transplant recipients experienced acute GVHD, with grade 4 GVHD in three patients. Together, the aforementioned studies seem to support the notion that NK cells promote GVHD. However, contradictory results were obtained from other studies where adoptive transfer of donor-derived NK cells into HSCT recipients can prevent the occurrence of GVHD in mouse and humans (104–107). In an MHC mismatched murine model (BALB/c→C57BL/6), IL-2-activated donor-derived NK cells were administered with allogeneic bone marrow cells and splenocytes (104). Mice receiving pre-activated donor-derived NK cells significantly delayed the onset of GVHD and prolonged the survival of mice. Consistently, these mice exhibited no infiltration of inflammatory cells with normal structure of gut (104). In accordance, another animal study by Song et al. has shown that single infusion of IL-12/IL-18- pre-activated donor NK cells one day 0 after HSCT has mitigated severe or mild aGVHD, and enhanced GVL effects (108).

In line with animal data, clinical results from a phase 1 clinical trial have shown that the infusion of high doses of ex vivo-membrane-bound interleukin 21 (mBL-21) expanded donor-derived NK cells is safe without adverse effects, without increased GVHD or high mortality (109). Therefore, early infusion of pre-activated donor-derived NK cells has the potential of prevention of GVHD. However, it should be taken into account that different strategy for the activation of donor-derived NK cells might bring different outcomes. Other important issues that should be considered are the infusion timing of NK cells, MHC/HLA matching degree between donors and recipients as well as the pretreatment strategy before HSCT.

Interestingly, NK cells can alleviate cGVHD by directly constraining recipient minor histocompatibility Ag (mHA)-triggered proliferation of donor-derived CD4+ T cells in a Fas-dependent manner (110). Evidence from Ruggeri L’s report has indicated that the KIR ligand incompatibility between donor and recipient might endow donor-derived NK cells to prevent the occurrence of GVHD, via direct depletion of recipient-derived antigen-presenting cells (APCs) (107). Clinical investigation on the early NK cell reconstitution in 82 patients following T cell-depleted allo-SCT have shown that NK cell number at day 14 after HSCT was inversely correlated with the incidence of grade II-IV aGVHD (111). Mechanistically, NK cells at day 14 produced high levels of IL-10 and showed upregulation of gene transcript of IL-10 compared with healthy individuals, suggesting that the regulatory phenotype might enable NK cells to suppress the development of GVHD (111).

Together, NK cells could prevent GVHD via (1) direct lysing of activated T cells; (2) indirect inhibition of T cell proliferation through depleting host APCs; (3) production of suppressive cytokines, such as IL-10 (Figure 3).

Only one clinical study by Munneke et al. have tried to elucidate the role of ILC1s in GVHD after HSCT (12). In the study, patients without developing aGVHD displayed increased proportions of skin-homing donor-derived ILC1s. Notably, following transplantation, patients with more severe GVHD exhibited fewer circulating ILC1s in PB, compared with healthy controls. Mobilization of ILC1s seemed to be associated with increased expression of CD69, CLA, and CCR10 which correlated with less severe progression of GVHD (12). However, the functionality of these aforementioned ILC1s was not determined in this study. Further question is whether skin-homing ILC1s alone can prevent the occurrence of GVHD? As we know, multiple organs, including GI tract, skin, lung, liver, and mouth, in recipients are targeted in GVHD, while ILC1s-expressing CLA and CCR10, which are skin homing markers, might only traffic to the skin. Therefore, further experiments where direct infusion of ILC1s into recipients with GVHD need to be taken and will.
FIGURE 3 | Role of ILCs in GVHD. NK cells can suppress GVHD via three main mechanisms, including direct lysis of activated T cells, indirect inhibition of T cell proliferation through depleting host APCs and production of suppressive cytokines, such as IL-10. ILC1 cells might migrate to the skin and alleviate cutaneous GVHD. Intravenous infusion of donor-derived ILC2 cells into ongoing GvHD mice can reduce the production of Th1 and Th17 cells while increasing the number of MDSCs via secreting IL-13. ILC3 cells play a protective role in GVHD. Recipient-derived ILC3 cells can alleviate pretreatment regimen-induced GI tract lesion via secretion of IL-22. Furthermore, these ILC3 cells can improve thymopoiesis in the hosts after HSCT.

be beneficial to the understanding of the role of ILC1s in the prevention of GVHD.

It has been shown that ILC2 cells in the lower GI tract but not in the lung are sensitive to conditioning treatment and exhibit a limited repopulation ability from donor bone marrow (112). Remarkably, a single infusion of donor-derived ILC2 cells at day 7 post-HSCT was shown to remain effective at reducing the severity and mortality of ongoing aGVHD in murine model. Intravenously infused ILC2 cells migrated to the GI tract, produced Th2 cytokines, limited inflammatory Th1 and Th17 cells, and induced myeloid-derived suppressor cells (MDSCs). IL-13 produced by ILC2 cells seemed to be involved in this process. Importantly, infusion of donor ILC2 cells did not affect the beneficial graft-vs.-leukemia (GVL) effect (106). Collectively, these data indicate that intravenously infused donor-derived ILC2 cells have the capacity to alleviate ongoing aGVHD without affecting the beneficial GVL effect in murine models (112). However, several questions still require further elucidation. For instance, how do intravenously infused donor-derived ILC2 cells migrate to the GI tract in the context of GVHD? Why do these cells not migrate to the lungs of recipients? Furthermore, how do these cells survive during the migration process? All these questions require further investigation.

The Role of Recipient-Derived ILCs in GVHD
An increasing body of evidence has indicated that ILC3 cells have the capacity to promote tissue repair. Under homeostatic circumstances, ILC3 cells can respond to environmental signals and maintain tissue homeostasis. In contrast, abnormal signals from infection or tissue damage can activate the ILC3 response (9, 113, 114). Therefore, in GVHD conditions, induction of regimen-induced tissue damage might cause a dysregulated ILC3 response.

In an animal model, a deficiency in recipient-derived IL-22 was shown to significantly increase the severity and mortality of GVHD (113). Furthermore, pretransplantation conditions increased the intestinal expression of IL-22 in recipients, which was mainly produced by recipient-derived CD45<sup>+</sup>C<sup>Di3</sup>−RORγt<sup>+</sup>NKp46<sup>+</sup>IL-7Rα<sup>+</sup>CCR6<sup>+</sup> ILCs. In accordance, IL-22 deficiency resulted in more severe epithelial damage during aGVHD and significant loss of intestinal stem cells. Taken together, these data suggest that loss of tissue-protective IL-22-producing ILCs in the intestines of recipients might be a pathological factor responsible for the GI tract lesions observed in aGVHD (113).

Recent work has shed light on the correlation between thymopoiesis and GVHD. Mice with GVHD after allo-HSCT exhibited a loss of intrathymic ILC3s, decreased intrathymic levels of IL-22 and impaired recovery of thymopoiesis. Not surprisingly, IL-22<sup>−/−</sup> mice that underwent transplantation showed an increased severity of GVHD-associated thymic injury. IL-22 receptor<sup>−/−</sup> recipient mice that underwent transplantation displayed increased numbers of cortical and medullary thymic epithelial cells (TECs). In accordance, administration of exogenous IL-22 after transplantation improved thymopoiesis and promoted the development of new thymus-derived peripheral T cells (115, 116). These findings encourage researchers to uncover what actually occurs after loss of ILC3s in the hosts induced by an induction regimen.
CONCLUDING REMARKS

Although studies on ILCs have become a focus of research in recent years, the precise role of ILCs in the pathogenesis of GVHD remains elusive. Many questions remain to be answered in the future. For instance, what is the precise role of ILC1 cells in the pathology of GVHD? Can intravenous infusion of ILC3 cells alleviate ongoing GVHD? Lastly, how do these cells migrate to the GI tract in recipients after intravenous transfer? How about the clinical application of ILC2 for the treatment of GVHD? A recent study identified a cell population–ILCregs (117). Like Tregs, ILCregs have the suppressive ability to curb ILCs. Therefore, the question remains whether ILCregs play a role in the pathogenesis of GVHD? Additionally, what is the interaction between ILCs and ILCregs at the onset of GVHD? These questions require further elucidation in future work.

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AUTHOR CONTRIBUTIONS

LS drafted the manuscript. AL, FZ, and QZ revised the manuscript. MJ and LC did the language editing. All authors read this manuscript and approved its submission.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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