Human Interleukin-15 Improves Engraftment of Human T Cells in NOD-SCID Mice

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Human nonobese diabetic-severe combined immune deficiency (NOD-SCID) mouse chimeras have been widely used as an in vivo model to assess human immune function. However, only a small fraction of transferred human T lymphocytes can be detected in human peripheral blood lymphocyte (huPBL)-NOD-SCID chimeras. To improve the reconstitution of human T lymphocytes in NOD-SCID mice, the use of recombinant human interleukin-15 (rhIL-15) as a stimulator of human lymphocytes was explored. Administration of rhIL-15 after transplantation of huPBLs into NOD-SCID mice increased reconstitution of human T lymphocytes in a dose-dependent manner, with an optimal dosage of 1 μg/mouse. The number of human T lymphocytes (HLA-ABC+ CD3+) in the lymphoid organs or tissue of rhIL-15-treated huPBL-NOD-SCID mice increased 11- to 80-fold, and phytohemagglutinin-induced T-lymphocyte proliferation and cytokine production were significantly enhanced. Additionally, although mature human cells have not been thought to enter the murine thymus, human T lymphocytes were detected in the huPBL-NOD-SCID thymus after rhIL-15 treatment. Thus, rhIL-15 can be used to optimize long-term peripheral T-cell engraftment in these human-mouse chimeras and may also be useful in clinical treatment of T-cell deficiencies.

The NOD/LtSz-prkdcscid/prkdcscid (nonobese diabetic-severe combined immune deficiency [NOD-SCID]) mouse has provided a useful model with which to examine normal human immune function and development in vivo (12, 14). NOD-SCID mice lack functional lymphoid cells and show little or no serum immunoglobulin (Ig) with age (46). The original study by Mosier et al. reported that human lymphocytes could be engrafted into SCID mice (30). In human peripheral blood lymphocyte (huPBL)-SCID/NOD-SCID chimeric mice, human T and B cells persisted for months and could be detected in the peritonea and peripheral lymphoid organs. The chimeras were capable of mounting antigen-specific secondary responses to various antigens after immunization (13, 28, 29, 32, 34–36).

However, a limitation of the huPBL-SCID/NOD-SCID model is the low level of huPBL engraftment (31, 47). This problem has made the analysis of antigen-specific cellular immune responses extremely difficult and limited the usage of this model in-depth studies. Various strategies have been explored to improve the efficiency of huPBL engraftment into huPBL-SCID/NOD-SCID mice, including an increase in the number of cells transferred (15, 30), pretreatment of the recipient mouse with low-dose irradiation (1, 37, 45), or elimination of mouse natural killer (NK) cells by anti-asialo-GM1 (2, 37, 45) or a combination of irradiation and anti-asialo-GM1 (4). All these protocols have shown only marginal effects if both the functions and distributions of transferred lymphocytes in lymphoid organs or tissues of huPBL-SCID/NOD-SCID mice were considered.

Regarding the efficiency of engraftment of huPBLs into SCID/NOD-SCID mice, a wide variety of stimulators of human hematopoiesis have also been tested in efforts to promote the engraftment of lymphocytes into huPBL-SCID/NOD-SCID mice; these stimulators include interleukin 6 (IL-6), IL-4, growth hormone, and chemokines (6, 10, 33, 48). IL-15 was originally isolated from culture supernatants of the simian kidney epithelial cell line CV-1/EBNA (11) and has been shown to have hematopoiesis-promoting effects, including development and differentiation of natural killer (NK) cells, proliferation of T cells, and maturation of B cells (7, 11, 22, 25, 42). A 4-day treatment with IL-15 in serum-free medium alone or synergically with IL-2 enhanced the cytotoxicity of human NK and LAK cells against tumor cells (23, 24). It was also found that IL-15 improved stem cell development in a semi-solid colony assay system (7). Those observations indicate that IL-15 is a strong immune hematopoiesis-promoting cytokine that may be an appropriate candidate for promoting transplantation of huPBLs into NOD-SCID mice. In this study, we have found that recombinant human IL-15 (rhIL-15) can be used for reconstitution of human T cells in NOD-SCID mice.

MATERIALS AND METHODS

Mice. NOD/LtSz-prkdcscid/prkdcscid mice were obtained from the Animal Production Area (Shanghai laboratory animal center, Chinese Academy of Sciences). Mice were not used until the age of 8 to 12 weeks. NOD-SCID mice were housed in microisolator cages, with all sterile food, water, and bedding. NOD-SCID mice received trimethoprim and sulfamethoxazole (40 mg/ml trimethoprim and 200 mg sulfamethoxazole per 320 ml water) in their drinking water and were kept under specific-pathogen-free conditions at all times.
**FIG. 1.** Protocol for examination of engraftment of huPBLs into NOD-SCID mice. huPBLs were separated by Ficoll density gradient centrifugation and washed twice with HBSS. After the cells were counted and adjusted to $1 \times 10^7$/ml, $5 \times 10^7/0.5$ ml of the purified huPBLs were transferred i.p. into recipient NOD-SCID mice that had been pretreated with irradiation. After transfer, the huPBL-NOD-SCID mice were injected i.p. either with the indicated amount of rIL-15 or with HBSS as a control every other day for a total of 10 injections starting on day 1.

**Creation of huPBL-NOD-SCID mice.** All donors for huPBLs were from the Anhui Provincial Blood Bank, were routinely screened for human immunodeficiency virus and hepatitis B virus, and provided informed consent before donation. The huPBLs were separated by Ficoll (Sigma, St Louis, MO) density gradient centrifugation following lysis of the red blood cells. The recovered cells usually were found to contain more than 90% lymphocytes when examined by lymphocyte counting. The mice received total-body irradiation at 3.0 Gy, followed by injection of $5 \times 10^7$ freshly isolated huPBLs intraperitoneally (i.p.) within 2 to 4 h after irradiation.

Mice were then injected i.p. with either the indicated amount of rIL-15 (Immunex, Seattle, WA) or Hanks’ balanced salt solutions (HBSS) as a control every other day for a total of 10 injections, starting on day 1. The protocol for rIL-15 or HBSS treatment and the point for the appropriate analysis are diagramed in Fig. 1.

**Preparation of mononuclear cells from immune organs of huPBL-NOD-SCID mice.** Under deep ether anesthesia, the mice were sacrificed by exsanguination from the subclavian artery and vein, and then the spleen, liver, lymph nodes, and thymus were removed and a femur was dissected. To obtain the mononuclear cells from peripheral lymphoid organs, the liver was pressed through a 200-gauge stainless-steel mesh and then suspended in HBSS. After incubation on ice for 20 min, the supernatant was centrifuged at 2,400 rpm for 30 min at 4°C. The interface cells between the Percoll solutions were aspirated and washed twice with HBSS. Splenocytes were passed through a 200-gauge stainless-steel mesh and were treated with erythrocyte lysis solution (155 mM NH₄Cl, 10 mM KHCO₃, 1 mM NaN₃). The cells were then resuspended in 40% Percoll (GIBCO-BRL Ltd.) solution containing 100 U/ml heparin, and the cell mixture was loaded on a layer of 70% Percoll solution and then centrifuged at 2,400 rpm for 30 min at 4°C. The interface cells between the Percoll solutions were aspirated and washed twice with HBSS. Lymphocytes, including mouse and human lymphocytes, were acquired in each run. For each mouse analyzed, cells were also stained with mouse IgG conjugated to FITC and PE as an isotype control. Fluorescence levels that excluded more than 98% of the cells in the negative controls were considered to be positive and specific for human staining. The cells were fixed in a 3% formalin–HBSS solution and stored at 4°C until flow cytometry analysis. Samples gated on the forward light scatter (FSC) and side light scatter (SSC) were used to identify viable lymphocytes. Proportions of the major subsets were determined by single and quadrant analysis. The percentage of Cy4-, FITC-, or PE-positive cells was measured by a FACScan using the CELLQUEST program (Becton Dickinson, San Jose, CA).

**Proliferation assay by [3H]thymidine incorporation.** From day 1 to day 20 after huPBL transfer, huPBL-NOD-SCID mice were injected i.p. with rIL-15 (1 µg/injection, every other day for a total of 10 injections). On day 28, both the cells of lymph nodes from huPBL-NOD-SCID mice and donor huPBLs were harvested and suspended in RPMI 1640 medium containing 10% fetal calf serum in 1 x 10⁶ human T cells/ml. The cells (1 x 10⁶/100 µl/well) were cultured in 96-well flat-bottom plates (Costar) with or without 10 µg/ml phytohemagglutinin (PHA) (Sigma). Three days later, the proliferation activity of lymph node cells was assessed by pulsing with 1 µCi (3.7 x 10⁶ Bq) of [3H]thymidine (6.7 Ci/mmol) (Shanghai Nuclear Technique Company of the Chinese Academy) for 8 h. The radioactivity of each sample was counted by a liquid scintillation counter (LS-6500; Beckman).

**Detection of cytokines.** The supernatants from the proliferation assay with PHA stimulation were collected at 18, 36, and 72 h. Then we measured the production of gamma interferon (IFN-γ) and IL-2 by the supernatants by a quantitation enzyme-linked immunosorbent assay (ELISA) according to the manufacturer’s instructions (RayBiotech, Inc.).

**Statistical analysis.** All experiments were performed at least three times. Student’s $t$ test was performed to determine the statistical difference. A $P$ value of $<0.05$ was considered significant.

**RESULTS**

IL-15 promoted the engraftment of human T cells into the thymuses and spleens of huPBL-NOD-SCID mice in a dose-dependent manner. A limitation of the huPBL-SCID/NOD-SCID model is the low level of huPBL engraftment (31, 47). Previous studies have demonstrated that rIL-15 promotes human T-cell proliferation and activation in vitro and in vivo, showing that IL-15 has hematopoiesis-promoting actions (11, 22, 25, 42). In this study, we aimed to investigate the role of IL-15 in T-cell engraftment using the huPBL-NOD-SCID model.

The irradiated recipient NOD-SCID mice were transplanted with $5 \times 10^7$ freshly purified huPBLs. To determine the optimal dosage of rIL-15, the huPBL-NOD-SCID mice re-
ceived different doses of rhIL-15—0.25, 0.5, 1, and 2 μg per mouse—and the control group received HBSS. The results had shown that 4 weeks after huPBL transplantation, the percentages of HLA-ABC + CD3+ T lymphocytes prepared from the thymuses and spleens of rhIL-15-treated huPBL-NOD-SCID mice had increased in a dose-dependent manner (Fig. 2). B-cell engraftment in the spleen after IL-15 treatment was also observed. Unexpectedly, splenic HLA-ABC+ CD19+ B cells showed no significant change after rhIL-15 treatment. These results suggest that IL-15 can enhance T-cell engraftment during huPBL transplantation. In the preliminary study, when mice received 10 μg rhIL-15 each, they manifested obvious skin lesions and other serious xenogeneic graft-versus-host disease (X-GVHD) symptoms. Therefore, we selected 1 μg of rhIL-15 per mouse as the optimal dosage for subsequent experiments.

**IL-15 enhanced human T-lymphocyte engraftment into lymphoid organs or tissues of NOD-SCID mice.** In order to further understand the engraftment-promoting effect of IL-15, we observed T-cell populations in other peripheral lymphoid organs or tissues. The results showed that IL-15 could increase the percentage of T cells not only in the thymus and the spleen but also in the liver, lymph nodes, and peripheral blood (Fig. 3A). With regard to the increase in the total number of lymphocytes in the thymus, spleen, liver, lymph nodes, and peripheral circulating blood during transplantation, the absolute number of human T cells was significantly increased about 80-fold in the thymus, 27-fold in the spleen, about 24-fold in the liver, 28-fold in the lymph nodes, and 11-fold in peripheral circulating blood in the rhIL-15-treated group (Fig. 3B). Simultaneously, the percentage and absolute number of T cells were dramatically decreased in the peritoneal cavity, suggesting that enhanced T-cell repopulation is due to the role of IL-15 in enhanced T-cell trafficking to lymphoid organs and tissues. To further address this issue, we observed the dynamics of T-cell numbers in three organs, the peritoneal cavity, the spleen, and the thymus, which had abundant T cells during the process of huPBL transplantation. As expected, T-cell numbers went up in the spleen and the thymus, along with a gradual decrease in the peritoneal cavity (Fig. 4). Taken together, rhIL-15 promoted the engraftment of human T cells into lymphoid organs or tissues of NOD-SCID mice, probably through its enhanced migration or trafficking.

**IL-15 treatment showed no difference in its effects on engraftment of CD4+ versus CD8+ cells.** Next, we investigated the effect of IL-15 on engraftment of T-cell subsets. As shown in Fig. 5, CD4+ and CD8+ cells account for 49% and 25% of donor huPBLs, respectively. Twenty-eight days after transplantation, both CD4+ and CD8+ T cells had repopulated the thymus, spleen, lymph nodes, liver, and peripheral blood well in rhIL-15-treated huPBL-NOD-SCID mice. The ratios of CD4+ to CD8+ cells in these organs or tissues were equal to those in donor huPBLs before transplantation. In summary, the engraftment-promoting effects of IL-15 on the CD4+ and CD8+ T-cell subsets were similar.

**Human T cells isolated from IL-15-treated huPBL-NOD-SCID mice had normal function.** In addition to the distribution and phenotype of engrafted huPBLs in rhIL-15-treated huPBL-NOD-SCID mice, the functional characteristics of huPBLs were also investigated. Lymphocytes were harvested from lymph nodes of huPBL-NOD-SCID mice that had been injected with rhIL-15. The human T-cell-specific mitogen response (PHA-stimulated proliferation) was tested. As shown in Fig. 6, the counts per minute increased significantly in lymphocytes from rhIL-15-treated huPBL-NOD-SCID mice after PHA stimulation. The supernatants of PHA-stimulated lym-
phocytes from rhIL-15-treated huPBL-NOD-SCID chimeras contained high concentrations of IFN-γ and IL-2 (Fig. 7A and B). There is no statistically significant difference in T-cell proliferation and cytokine production between engrafted T cells isolated from IL-15-treated huPBL-NOD-SCID mice and human donor PBLs, suggesting that the former function normally, like freshly isolated huPBLs.

**DISCUSSION**

Reports have been accumulating from studies examining the role of IL-15 in immune function (3, 7, 9, 22–25, 42). A high-affinity receptor for IL-15 has been described on human B cells, T cells, and NK cells, and activation of these cells results in a further induction of IL-15 receptor (IL-15R) expression (3, 25). IL-15R has been shown to play a critical role in IL-2-induced T-cell proliferation in vitro (41). A defect in the IL-15R gamma chain leads to a significant loss of thymic lymphocytes in SCID mice (43). Recently, it was reported that IL-15 exhibited a dose-dependent enhancement of murine IgM and IgG secretion from B cells that were treated with anti-IgM and IL-2 or anti-IgM alone in vitro and in vivo (5, 17, 40). Furthermore, IL-15R has been found on NK cells (8, 39, 49, 51), and IL-15 was able to increase the proliferation response and cytotoxicity of NK cells against sensitive or nonsensitive tumor cells in humans, mice, and rats, either independently or synergically with IL-2 (19, 21, 50, 52–54). It is also noted that rhIL-15 improved the differentiation and engraftment of human NK cells from human hematopoietic stem cells in NOD-SCID mice (18).

Here we reported that the use of rhIL-15 could significantly improve the engraftment and reconstitution of human T lymphocytes in NOD-SCID mice after transfer of human peripheral blood mononuclear cells. Significantly larger amounts of human T lymphocytes were found in the thymuses of rhIL-15-treated mice than in those of untreated control mice. The thymus differs from other lymphoid organs because it manifests more restricted cell entry, leading to more difficult trafficking to the murine thymus due to a lack of appropriate
adhesion molecules (26). These results suggested a pivotal role of IL-15 in normal T-lymphocyte development, which allowed thymus entry of human lymphocytes and promoted peripheral localization of the mature T cells from the thymus to the spleen, lymph nodes, and liver (Fig. 3). The lymphoid liver is a newly emerging concept that has been a hot spot for immunologists, and the characterization of the liver as a lymphoid organ has been discussed in several reviews (20, 27, 38). Meanwhile, we investigated the distribution of human lymphocytes in nonlymphoid organs such as the small intestine and lung; the results showed that there were no human lymphocytes in these organs.

FIG. 4. rhIL-15 promoted the trafficking of human T cells to lymphoid organs of NOD-SCID mice. To prove the promoting role of IL-15 in T-cell trafficking to lymphoid organs and tissues, we collected the mononuclear cells from the peritoneal cavities, spleens, and thymuses of rhIL-15-treated huPBL-NOD-SCID mice on days 7, 14, 21, and 28 after huPBL transfer, stained them with PE-labeled anti-HLA-ABC and Cy-labeled anti-CD3, and then analyzed them by flow cytometry. Data are representative of three independent experiments, with similar results obtained in each experiment.

FIG. 5. Flow cytometry analysis of human T-lymphocyte subsets from lymphoid organs or tissues of huPBL-NOD-SCID mice. To test human T-lymphocyte subsets from lymphoid organs or tissues of huPBL-NOD-SCID mice, on day 28, the lymphoid organs or tissues of huPBL-NOD-SCID mice and donor huPBLs were collected, stained with Cy-labeled anti-CD3, FITC-labeled anti-CD4, and PE-labeled anti-CD8 antibodies, and then analyzed by flow cytometry. The analysis was performed on gated lymphocytes with FSC/SSC characteristics. Results are expressed as means ± standard deviations for three independent assays. *P < 0.001 for comparison of the rhIL-15 group with the HBSS group.

FIG. 6. Proliferation of the cells of lymph nodes from rhIL-15-treated huPBL-NOD-SCID chimeras stimulated with PHA in vitro. On day 28 after huPBL transfer, the cells of lymph nodes of rhIL-15-treated mice and donor huPBLs were harvested. All cells (1 × 10^5 human T cells/100 μl/well) were cultured in 96-well flat-bottom plates with or without 10 μg/ml PHA. Three days later, proliferation activity was assayed by pulsing with 1 μCi (3.7 × 10^7 Bq) of [3H]thymidine (6.7 Ci/mmol) for 8 h; the radioactivity for each sample was counted by a liquid scintillation counter. Data are presented as the means ± standard deviations of triplicate samples. There was no obvious difference between donor T cells and rhIL-15-treated chimera human T cells in response to PHA. *P < 0.001 for comparison of the lymphocytes of the rhIL-15-treated chimera with and without PHA.
organs in huPBL-NOD-SCID mice (data not shown). All these findings meant that there was a selective migration of huPBLs into lymphoid organs or tissues. We also showed that differences in the background numbers of human CD3\(^+\) T cells in lymphoid organs or tissues appeared to be donor dependent. Different donors generally have different background migration. This may be due to the activation state, phenotype, or adhesive ability of the donor T cells.

Reportedly, IL-15 plays a major role in maintenance of CD8\(^+\) memory T cells (16, 44), but our data showed no obvious difference in the effects of IL-15 on the engraftment of CD4\(^+\) versus CD8\(^+\) T cells into NOD-SCID mice. The differences may result from the difference in the dosage of rhIL-15 used. It is guessed that IL-15 at a high dose may preferentially promote CD8\(^+\) T-cell engraftment.

Our test also revealed that the dosage of rhIL-15 was very important to the effect on the constitution of huPBL-NOD-SCID mice. In our preliminary investigations, when mice were injected i.p. with 10 \(\mu\)g rhIL-15 per mouse every other day for a total of 10 times after huPBL engraftment, we found that serious X-GVHD occurred, and almost all of the mice had wasting and skin lesions and finally died. In view of severe GVHD symptoms at high doses, we chose 1 \(\mu\)g as a rational dose in that the effectiveness of T-cell engraftment at 1 \(\mu\)g was similar to that at 2 \(\mu\)g; moreover, mice that received 1 \(\mu\)g IL-15 showed no GVHD symptoms.

Moreover, when rhIL-2 was used as a positive control in the treatment of huPBL-NOD-SCID mice in our study, we found that rhIL-15 had still better abilities to promote engraftment and reconstitution of human T lymphocytes in NOD-SCID mice than rhIL-2 (data not shown), although rhIL-2 showed limited promoting activities. The proliferation of T cells and cytokines secreted by rhIL-15-treated huPBL-NOD-SCID mice was significantly enhanced and was not obviously different from that of donor human T cells when the cells were stimulated with PHA (Fig. 6 and 7), indicating that the engrafted human T lymphocytes have normal function and that this kind of model can be used in future work.

In our experiments, 1 month after adoptive transfer, human B cells were found mainly in the peritoneal cavity, but that only accounted for approximately 1 to 5% of the total cells. It is possible that these B cells migrated to lymphoid organs if they did not die in situ. Human B cells were only occasionally observed in secondary lymphoid tissues, such as the spleen and lymph nodes, in huPBL-NOD-SCID mice. Flow cytometry analysis indicated that there was no significantly greater engraftment of HLA-ABC\(^+\) CD19\(^+\) cells in lymphoid organs of rhIL-15-treated mice than in the control. However, high levels

![Graph A](image1.png)

![Graph B](image2.png)

**FIG. 7.** Cytokine production by the cells of lymph nodes from huPBL-NOD-SCID mice treated with rhIL-15. The cells of lymph nodes from rhIL-15-treated mice and donor huPBLs were used in this experiment. The cells were cultured in 96-well flat-bottom plates with or without 10 \(\mu\)g/ml PHA in vitro. At 18, 36, or 72 h, as indicated, cell culture supernatants were collected for determination of IFN-\(\gamma\) (A) or IL-2 (B) by quantitation ELISA. Data are presented as the means ± standard deviations of triplicate samples. There was no obvious difference in cytokine production between donor T cells and rhIL-15-treated chimera human T cells. \(P < 0.001\) for comparison of the lymphocytes of the rhIL-15-treated chimera at different time points with and without PHA.
of human immunoglobulin were observed in sera of rhIL-15–treated huPBL-NOD-SCID mice, indicating that human B cells must be present and functional in these mice, though it may be difficult to define the phenotype and quantities of human B cells in the mice.

Our findings suggest that rhIL-15 has clinical applications in immune deficiency diseases or bone marrow transplantation, where it might be advantageous to accelerate peripheral expansion and to promote localization of T cells. The molecular mechanisms underlying the effects of rhIL-15 in huPBL-NOD-SCID mice are under investigation.

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