Human Prolactin Improves Engraftment and Reconstitution of Human Peripheral Blood Lymphocytes in SCID Mice

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Recombinant human prolactin (rhPRL) was administered to huPBL-SCID mice to determine its effects on human immunologic reconstitution and function. The huPBL-SCID mice were given 10 μg i.p. injection of rhPRL every other day for a total of 10 injections after huPBL were transfered. The results demonstrated that rhPRL improved the engraftment of lymphocytes into thymus, lymph nodes and spleens, showing that the cellularities of these organs increased although the cellularities tended to vary depending on the donor. The amounts of human T cells (HLA-ABC$^+$/CD3$^+$) increased greatly in thymus (14.2 folds), spleen (4.16 folds) and lymph nodes (40.18 folds) after rhPRL injections. The amounts of human B cells (HLA-ABC$^+$/CD19$^+$) also increased greatly in lymph nodes (42.5 folds) and spleen (5.78 folds). The lymph node cells from the rhPRL-treated huPBL-SCID mice were more sensitive to PHA stimulation ([^{3}H] thymidine incorporation). The supernatant of PHA-stimulated PBL from rhPRL-treated huPBL/SCID chimerism contained more cytokines (IFN-γ and IL-2). The natural cytotoxicity against human sensitive target cells, K562 cells, from spleen and bone marrow of hPBL/SCID chimerism was significantly enhanced by rhPRL administration. The lymph node cells were stimulated with LPS *in vitro* for 3 days and the lymphocytes from the rhPRL-treated huPBL-SCID mice were more sensitive to mitogen stimulation. Both serum total IgG level and IgM level of rhPRL-treated huPBL/SCID chimerism were increased, and even without DT-rechallenge the base line of DT-specific IgG was elevated after rhPRL treatment in huPBL-SCID mice. Thus, rhPRL stimulation promotes reconstitution of human immune system in huPBL-SCID mice. *Cellular & Molecular Immunology*. 2004;1(2):129-136.

Key Words: human/mouse chimera, prolactin, B cell, T cell

Introduction

Prolactin, initially described as a peptide hormone secreted by the anterior pituitary, has been shown to exert a variety of biological effects *in vivo* and has been suggested to also exert effects on hematopoietic and immune cell types. It is known that differentiation and development of various blood cell lineages from hematopoietic progenitor cells and coordination of host immune response are regulated by a group of proteins known as cytokines. The receptors for these factors have been grouped in a large family of structurally-related molecules including IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, GM-CSF, G-CSF, M-CSF, erythropoietin, prolactin (PRL) and growth hormone (GH), which bind to distinct but related receptors (1). Because of the structural similarities between adenopituitary hormones (GH and PRL) and conventional hematopoietic cytokines, GH and PRL, recently have been shown to exert many immuno-hematopoietic-promoting effects (2). Specific depression of PRL release induced by bromocriptin is associated with decreased T cell function and the response of T cells *in vitro* is depressed in the presence of anti-PRL antibodies (3, 4). PRL increased the proliferating response of NK, T, and B cells to mitogenic stimuli such as IL-2, PHA and SAC respectively (5). A 4-day treatment with PRL in serum-free medium independently or synergically with IL-2 enhanced the cytotoxicity of human NK and LAK to sensitive or nonsensitive tumor targets (6). It was also found that PRL improved the stem cell development in semisolid colony assay system (7). We have found that PRL administration increased the antigen-specific proliferation of lymph node T cells of both normal and dwarf mice (8). However, most of the experiments were made *in vitro* or in animal and may need further study *in vivo* or in human.

The engraftment of normal human lymphocytes into mice offers an involuable means to examine normal human

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Abbreviations: SCID, severe combined immune deficiency; huPBL, human peripheral blood lymphocytes; rhPRL, recombinant human prolactin; DT, diphtheria tetanus.

immune function and development in an *in vivo* setting. Mice with severe combined immune deficiency (SCID) have a defect in their recombinase system which does not allow for the productive rearrangement of their immune receptor genes (9). These mice lack mature T and B cell function and are incapable of rejection solid tissue graft. The original report by Mosier et al. described the ability of human lymphocytes to engraft into SCID mice (10). The huPBL were injected intraperitoneally and human T and B cells persisted in these mice for months and could be detected in the peritoneum and peripheral lymphoid organs of the mice, and are capable of mounting antigen-specific secondary responses to various antigens after immunization (11).

Here we want to assess the effects of rhPRL treatment on reconstitution of human immune system including T lymphocytes, B lymphocytes and NK cells in huPBL-SCID mice. We report here that rhPRL can promote human lymphocyte engraftment into peripheral lymphoid organs and improve their function in this human/mouse model.

Materials and Methods

Mice

C.B-17 SCID mice were obtained from the Animal Production Area (NCI-FCRDC, Frederick, MD). Mice were not used until 8-12 weeks of age. SCID mice were housed in microisolator cages, and all food, water, and bedding were autoclaved before use. SCID mice received trimethoprim and sulfamethoxazole (40 mg/ml trimethoprim and 200 mg sulfamethoxazol) in suspension in their drinking water and were kept under specifipathogen-free condition all the time.

Creation of huPBL-SCID mice

All donors for huPBL were screened for HIV-1 and hepatitis B and provided informed consent before donation. The huPBL were purified by counter-current elutriation and lymphocyte fraction, containing >90% lymphocytes were obtained. All mice received 20 μl of αASGM1 (wako Chemicals, Dallas, TX) i.v. the day before injection of huPBL, which we have demonstrated to improve egraftment by the removal of host NK cells (12). The huPBL (1×10^8) were injected i.p. into recipient SCID mice. Each experiment used huPBL from a different donor and 4-6 mice per group. Mice received either 10 μg of rhPRL (provided by Genezyme Corporation, Framingham, MA) or HBSS i.p. every other day for a total of 10 injections starting at day 1 (Figure 1). Blood samples were collected before every immunization to determine the amount of human DT-specific antibodies or total immunoglobulin using ELISA.

ELISA for DT-specific antibodies

A 96-well round-bottom plate with 100 μl/well of a mixed solution containing both DTX and TTX at a final concentration of 100 ng/well was placed overnight at 4°C. The plate was then washed 3 times and allowed to dry. The plates were then given 200 μl of a blocking buffer for 2 hours. The blocking buffer consisted of PBS with 5% filtered chicken serum. After washing, the test samples, including a normal human serum control, were diluted at 1:10, 1:100, and 1:1000 with PBS. Each sample (100 μl) was placed in the wells. The plates were incubated for 2 hours at 37°C, then washed again and a mix of goat anti-human IgG and IgM (alone or together)-alkaline phosphatase (100 μl) (Sigma) was added to the wells and incubated for 1 hour at room temperature. The plates were washed again and a phosphatase substrate tablet solution (Sigma) was added at 100 μl/well and the color was allowed to develop until the positive control reached an optical density (OD) close to 1.0. The plates were then read at 450 nm. Data are presented as OD using a dilution of serum of 1:10 or 1:100. Since there was no positive control monoclonal antibody to DT available to do a standard curve, the data must be presented as OD value.

ELISA assay for cytokines

The supernatants of PHA-stimulated MNCs were collected at the 18th, 36th and 72th hour and stored at -70°C for ELISA assays. Following the protocol of the kits, the cytokines IFN-γ (detection limit, 50 pg/ml) and IL-2 (detection limit, 8 pg/ml) were checked by ELISA kits (R&D Systems). The data were analyzed with software Origin Pro 7.0. The experiment was repeated for three times.

Flow cytometry analysis (FCM)

Splenic, thymic and lymph node (pooled from mesenteric, axillary, and inguina) were harvested at day 28 after huPBL transfer and single-cell suspensions were prepared, blocked, stained, and analyzed by double-color FCM analysis as previously described (12). Reagents used included FITC-labeled anti-HLA-ABC and PE-labeled anti-CD3 or CD19. All antibodies were obtained from Becton Dickinson. The cells were fixed in 100% paraformaldehyde and analyzed on an EPICS flow cytometer. Each fluorescence study had directly-labeled double-negative isotype controls of normal rat immunoglobulin.

Mitogen assays of lymph node cells

At day 1 after huPBL transfer, the huPBL-SCID mice received rhPRL injection i.p. (10 μg/injection, every other day for total 10 injections). At day 28, the lymph node cells were harvested and the cell suspension was exposed to mitogen (PHA or LPS assay). PHA (10 μg/ml, Sigma) or LPS (10 μg/ml, Sigma) and lymph node cells (1×10^6/200 μl/well) was added to flat-bottomed 96-well plate (Costar). Three days (for mitogen assay) or five days (for DT-specific proliferation) later, proliferation was assayed by pulsing with 1mCi (3.7×10^4 becquerels) of [^{3}H] thymidine (6.7 Ci/mmol) (New England Nuclear, Boston, MA) for 8h and harvested with MASH II apparatus (Microbiological Associates, Bethesda, MD). A Student's *t*-test was performed to determine statistical difference with $p < 0.05$ being considered significant.

Cytotoxicity assay

Cytotoxic activity of NK cells in PBMCs was assessed against ^{51}Cr-labeled K562 in a standard 4h ^{51}Cr-release assay. K562 cells (1×10^6) were labeled with 50 μCi of ^{51}Cr for 1h at 37°C and washed three times. Effector (PBMC)

Table 1. *RhPRL improves human lymphocyte engraftment into human PBL/SCID chimeras.*

	Cellularity ($\times 10^6$)	$ABC^+/CD3^+$		$ABC^+/CD19^+$	
		%	Amount ($\times 10^4$)	%	Amount ($\times 10^4$)
Thymus					
HBSS	0.74 ± 0.25	1.34 ± 0.92	1.02 ± 0.46		
rhPRL	1.37 ± 0.33	10.49 ± 3.37	14.46 ± 5.08*		
Lymph Node					
HBSS	0.36 ± 0.15	0.84 ± 0.31	0.34 ± 0.17	0.43 ± 0.12	0.16 ± 0.06
rhPRL	1.65 ± 0.62	8.40 ± 4.99	13.66 ± 6.11**	3.78 ± 1.89	6.80 ± 2.55**
Spleen					
HBSS	9.75 ± 2.85	0.72 ± 0.23	7.02 ± 2.95	0.58 ± 0.17	5.56 ± 3.03
rhPRL	21.41 ± 4.73	1.37 ± 0.34	29.24 ± 9.78**	1.50 ± 0.43	32.12 ± 10.14*

HuPBL-SCID mice were created in Materials and Methods. RhPRL (10 μg) or HBSS were injected i.p. every other day for total 20 days. At day 28 after huPBL transfer, the thymus, spleen and lymph node (axillary, inguinal and mesenteric) were harvested, stained by flueorecence-labelled antibodies and analyzed by flowcytometry as described in Materials and Methods. Representative of two to three experiments with three to five mice/group. *p<0.05, ** p<0.01 compared rhPRL with HBSS group.

and target (K562) were incubated for 4h at the E:T of 12.5:1, 25:1, 50:1, 100:1. Spontaneous release of ^{51}Cr was determined by incubating the target cells with medium alone and was always less than 10%. Maximum release was determined by adding SDS to a final concentration of 5%. The percentage ^{51}Cr release was calculated as follow: 100×[(experimental release-spontaneous release)/(maximum release-spontaneous release)]. Each experiment was done twice.

Statistical analysis

All experiments were performed at least 3 times and had at least three mice per group, with a representative experiment being shown. A Student's *t*-test was performed to determine if values differed significantly ($p < 0.05$ or $p < 0.01$).

Results

Recombinant human PRL improves human lymphocytes engrafting into SCID mice

Human PBL was transferred into SCID recipients in order to determine if the rhPRL increased peripheral engraftment in mice. All SCID recipients were first treated with αASGM1, which we have previously found to improve human cell engraftment through the removal of host NK cells (11). Some of the SCID recipients received 1×10^8 huPBL followed by either recombinant human prolactin (rhPRL, 10 μg i.p. every other day for 20 days) or HBSS alone. Four weeks after cell transfer, various lymphoid organs, including murine thymus, of the SCID recipients were removed and analysed for the presence of human cells as detected by flow cytometric analysis (Figure 1). The results demonstrated that rhPRL improved the engraftment of lymphocytes into thymus, lymph nodes and spleens, showing that the cellularities of these organs increased (Table 1) although the cellularities tended to vary depending on the donor. The amounts of human T cells ($HLA\text{-}ABC^+/CD3^+$) increased greatly in thymus (14.2 folds), spleen (4.16 folds) and lymph nodes (40.18 folds) after rhPRL injections (Table 1). The amounts of human B cells ($HLA\text{-}ABC^+/CD19^+$) also increased greatly in lymph nodes (42.5 folds) and spleen (5.78 folds) (Table 1). The results suggested that rhPRL promoted the human lymphoid cell development in SCID mice, showing a greater incidence and degree of engraftment of human cells in the peripheral lymphoid organs, including the murine thymus.

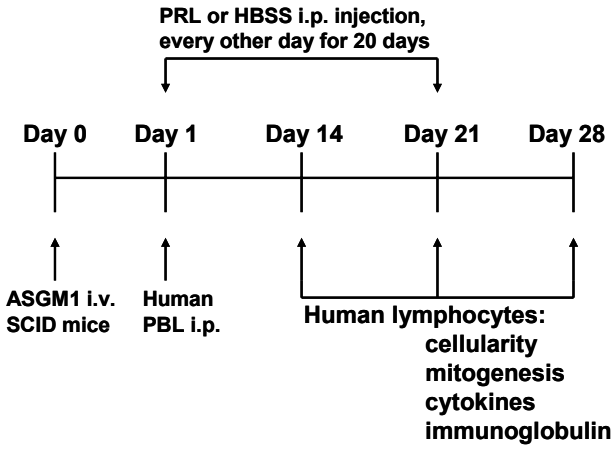

Figure 1. Protocol for examination of engraftment of human PBL into SCID mice. Human PBL were purified by counter-current elutriation and lymphocyte fraction, containing >90% lymphocytes were obtained. All mice received 20 μl of αASGM1 (wako Chemicals, Dallas, TX) i.v. the day before injection of huPBL. The huPBL (1×10^8) were injected i.p. into recipient SCID mice. Each experiment used huPBL from a different donor and 4-6 mice per group. Mice received either 10 μg of rhPRL or HBSS i.p. every other day for a total of 10 injections starting at day 1.

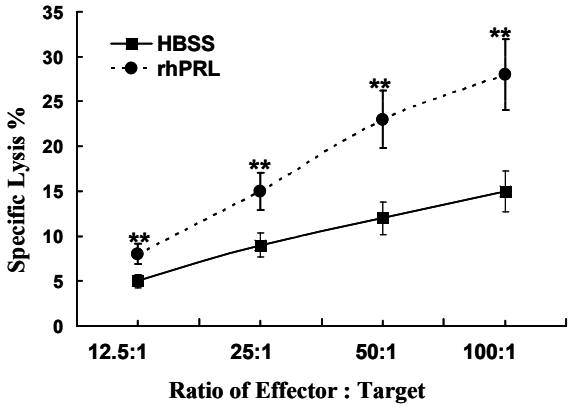

Figure 2. Enhanced natural cytotoxicity against K562 cells by rhPRL injections. The cytotoxicity of human NK cells against K562 cells was examined by ^{51}Cr 4-hour release assay as described in Materials and Methods. The cells were co-cultured with ^{51}Cr-labelled K562 cells in each ratio of effector/ target (E:T) for another 4 hours. Each test was divided into control group and rhPRL treatment group. Four replicate wells were used. After standard 4h incubation, the supernatant was harvested and analyzed on a γ-counter. The % specific lysis was calculated as described in Materials and Methods. RhPRL significantly improved the cytotoxicity of human NK cells against K562 cells (**p<0.01). Each group has three mice and the experiments were repeated three times.

Table 2. *Proliferation response of human PBL from huPBL/SCID chimerism to mitogen in vitro.*

Group	PHA	[^{3}H]Thymidine uptake (cpm)
PBL	-	1,965 ± 510
PBL+rhPRL	-	2,042 ± 73
PBL	+	27,634 ± 1,058
PBL+rhPRL	+	53,208 ± 1,848**

At day 1 after huPBL transfer, the huPBL-SCID mice received rhPRL injection i.p. (10 μg /injection, every other day for total 10 injections). At day 28, the lymph node cells were harvested and the cell suspension was stimulated by mitogen. PHA (10 μg/ml, Sigma) and lymph node cells (1×10^6/200 μl/well) were added to flat-bottomed 96-well plate (Costar). Three-day proliferation was assayed by pulsing with 1 mCi (3.7×10^4 becquerels) of [^{3}H]thymidine (6.7 Ci/mmol) (New England Nuclear, Boston, MA) for 8h and harvested with MASH II apparatus (Microbiological Associates, Bethesda, MD). A Student's t-test was performed to determine statistical difference **p<0.01 compared rhPRL with control group.

Table 3. *Human cytokines in PHA-stimulated MNCs from huPBL/SCID chimeras.*

Group	HBSS	PHA
IFN-γ (pg/ml)		
HBSS	< 1	2,264 ± 317
PRL	< 1	28,524 ± 1,536
IL-2 (pg/ml)		
HBSS	< 1	8,530 ± 582
PRL	< 1	15,127 ± 625**

At day 1 after huPBL transfer, the huPBL-SCID mice received rhPRL injection i.p. (10 μg /injection, every other day for total 10 injections). At day 28, the lymph node mononuclear cells were harvested and the cell suspension was stimulated by mitogen (PHA, 10 μg/ml, Sigma). Lymph node cells (1×10^6/200 μl/well) were added to flat-bottomed 96-well plate (Costar) and then co-cultured with PHA. The supernatants of PHA-stimulated MNCs were collected at the 18th, 36th and 72th hour and stored at -70°C for ELISA assays. Following the protocol of the kits, the cytokines IFN-γ (detection limit, 50 pg/ml) and IL-2 (detection limit, 8pg/ml) were checked by ELISA kits (R&D Systems). The data were analyzed with software Origin Pro 7.0. The experiment was repeated for three times. A Student's t-test was performed to determine statistical difference **p<0.01 compared rhPRL with control group.

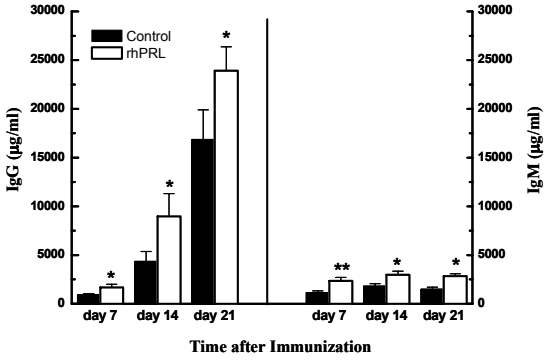

Figure 3. Effects of rhPRL on the production of total antibodies (IgG and IgM) without antigen rechallenging in huPBL-SCID mice. HuPBL-SCID mice were created as described in Materials and Methods. RhPRL (10 μg) or HBSS was injected i.p. every other day for total 20 days. At day 28 after huPBL transfer, the total serum IgG and IgM were analyzed. The experiment shown had 5-7 mice per group. The results are expressed in optical density units as assessed on a spectrophotometer at 450 nm from 1:100 dilution of each sample. RhPRL significantly (p<0.05) promoted the production of IgG and IgM even without DT-immunization.

RhPRL improves the function of human T lymphocytes and NK cells in huPBL-SCID mice

Although the cellularities and phenotypes of engrafted human lymphocytes are important to exhibit the function of rhPRL, we were more interested in the general function of the engrafted human lymphoid cells after *in vivo* treatment with rhPRL. The human T-cell specific mitogen (PHA-stimulated proliferation) response was introduced. Because there was no mitogen (e.g. PHA) response present in SCID mice, the PHA-stimulated proliferation is specific to the engrafted human lymphocytes. The lymph node cells were harvested from huPBL-SCID mice, in the presence or absence of rhPRL, 28 days after huPBL transfer. The cells were stimulated with PHA *in vitro* for 3 days. As shown in Table 2, the lymphocytes from the rhPRL-treated huPBL-SCID mice were more sensitive to mitogen stimulation, showing that the [^{3}H]thymidine incorporation after PHA stimulation significantly increased, indicating that rhPRL promoted the functional development, in addition to the engraftment, of human lymphocytes *in vivo*. The super-

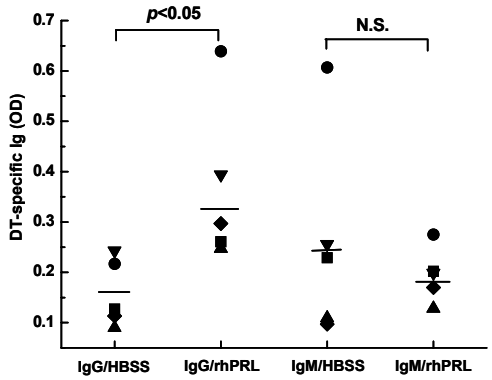

Figure 4. Effects of rhPRL on the production of DT-specific antibodies (IgG and IgM) without rechallenging with DT in huPBL-SCID mice. HuPBL-SCID mice were created as described in Materials and Methods. RhPRL (10 μg) or HBSS was injected i.p. every other day for total 20 days. At day 28 after huPBL transfer, the serum was analyzed using DT-specific ELISA assay as described in Materials and Methods. The experiment shown had 5-7 mice per group. The results are expressed in optical density units as assessed on a spectrophotometer at 450 nm from 1:100 dilution of each sample. RhPRL significantly ($p<0.05$) promoted the DT-specific IgG production even without DT-immunization.

natant of PHA-stimulated PBL from rhPRL-treated huPBL/SCID chimerism contained more cytokines (IFN-γ and IL-2), mainly produced by T cells and NK cells, than their control (HBSS-treated group) (Table 3). Importantly, the natural cytotoxicity against human sensitive target cells, K562 cells, from spleen and bone marrow of hPBL/SCID chimerism was significantly enhanced by rhPRL administration (Figure 2), indicating that human NK cells were improved to exert stronger function in huPBL/SCID chimerism by rhPRL.

RhPRL improves the function of human B lymphocytes in huPBL-SCID mice

The human B-cell specific mitogen (LPS-stimulated proliferation) response was introduced. Because there is no mitogen (e.g. LPS) response present in SCID mice, the LPS-stimulated proliferation is specific to the engrafted human lymphocytes. The lymph node cells were harvested from huPBL-SCID mice, in the presence or absence of rhPRL, 28 days after huPBL transfer. The cells were stimulated with LPS *in vitro* for 3 days. As shown in Table 4, the lymphocytes from the rhPRL-treated huPBL-SCID mice were more sensitive to mitogen stimulation, showing that the [^{3}H]thymidine incorporation after LPS stimulation significantly increased, indicating that rhPRL promoted the functional development, in addition to the engraftment, of human B lymphocytes *in vivo*. Meanwhile, we examined the serum total immunoglobulin levels to see the functional potential of engrafted B lymphocytes. As shown in Figure 3, both total IgG level and IgM level of rhPRL-treated huPBL/SCID chimerism were increased, indicating rhPRL improved the baseline level production of antibodies from human body which is critical in humoral immune response. In addition to observing the effects of rhPRL on human general immune function in huPBL-SCID mice, it is more

Table 4. *Proliferation response of human PBL to mitogens in vitro.*

Group	LPS	[^{3}H]Thymidine uptake (cpm)
PBL	-	2,133±532
PBL+ rhPRL	-	2,021±463
PBL	+	8,735±518
PBL+ rhPRL	+	14,707±639**

At day 1 after huPBL transfer, the huPBL-SCID mice received rhPRL injection i.p. (10 μg/injection, every other day for total 10 injections). At day 28, the lymph node mononuleic cells were harvested and the cell suspension was stimulated by mitogen (LPS, 10 μg/ml, Sigma). Lymph node cells (1×10^6/200 μl/well) was added to flat-bottomed 96-well plate (Costar) and then co-cultured with LPS. Three days proliferation was assayed by pulsing with 1 mCi (3.7×10^4 becquerels) of [^{3}H]thymidine (6.7 Ci/mmol) (New England Nuclear, Boston, MA) for 8 h and harvested with MASH II apparatus (Microbiological Associates, Bethesda, MD). A Student's *t*-test was performed to determine statistical difference ** $p<0.01$ compared rhPRL with control group.

important to see if rhPRL may improve human specific immune response. Based on the established huPBL-SCID mice model and results that rhPRL improved the engraftment of human lymphocytes, we further examined the serum baseline antibody against DT antigens (a vaccine mixture of the diphtheria toxin and tetanus toxin for human immunization in childhood) in huPBL-SCID mice in order to determine the possible regulation of rhPRL on human secondary specific immune response. It was noted that even without DT-rechallenge the base line of DT-specific IgG was elevated after rhPRL treatment in huPBL-SCID mice (Figure 4), suggesting that rhPRL improved the specific immune response although it was very weak without antigen rechallenge.

Discussion

Prolactin (PRL) is a 24 kD single chain hormone secreted by the anterior pituitary. While PRL primarily exerts its effects on lactation and reproduction, there has been a considerable amount of literature examining the role of PRL on immune function (13-16). High affinity receptor for PRL has been described on human B and T cells by direct binding of PRL, and activation of these cells resultes in a further induction of receptor expression (17). PRL receptors have been determined to play a critical role in IL-2 induced T-cell proliferation *in vitro* (18); PRL and corresponding mRNA are present in human T- and B-lymphocytes as well as in B-lymphoblastoid line and T leukemia cells, which has been suggested to act as an autocrine growth factor for lymphoid cell proliferation (19-25). It has been reported that PRL administration decreased thymic cellularity when examining the effects of ovine PRL in dwarf mice (26). Recently, it was reported that PRL exhibited a dose-dependent enhancement upon both IgM and IgG secretion from B cells treated with anti-IgM and IL-2 or anti-IgM alone *in vitro* (27, 28), indicating that PRL may affect peripheral B-cell function. PRL receptors are present in the NK cells (29). PRL increased

the proliferating response and cytotoxicity to sensitive or insensitive tumor of human, murine or rat NK cells independently or synergically with IL-2 targets (30-35). The signal pathways, such as MGF-STAT5, MAP kinase, JAK2 and ZAP-70, have been found on NK cells through prolactin receptor, which addressed the molecular actions of prolactin on NK cells (36-41). However, thus far there has been little evidence to indicate that PRL can affect engraftment of human immune cells *in vivo*.

Here, we first report that engraftment and reconstitution of human immune system in SCID mice may be improved by rhPRL. We and our collaborators have reported previously that PRL accelerates hematopoietic and immunologic reconstitution after autologous BMT to shorten the period of bone marrow aplasia and immunodeficiency (42-48). Ovine PRL could augment peripheral immune function in pituitary hormone-deficient dwarf mice and PRL administration increased numbers of hematopoietic progenitors in mice stressed by azido-thymidine, a myelotoxic drug. The results indicate that administration of rhPRL to mice after BMT enhances hematopoietic reconstitution as determined by increased hematopoietic progenitor cell content of bone marrow and spleen, and numbers of granulocytes, T-cell progenitors, and B-cell progenitors by flow cytometric phenotypic analysis, accelerated recovery of red blood cells in the peripheral blood, and improved splenic T-cell and B-cell mitogen responses. GH and PRL exerted differential effects on murine T-cell development and function in neuroendocrine hormone-deficient dwarf mice, which have been reported to have deficiencies in T-cell development and function. In contrast to the thymopoietic effects of GH, ovine PRL administration resulted in accelerated thymic atrophy in these mice. Interestingly, PRL administration had no effect on thymic size in normal mice and thymic recovery was more obvious at the 50 μg PRL dose than the 10 μg dose, suggesting that lack of other hormones in the dwarf mouse made it susceptible to the inhibitory effects of PRL. We speculate that there are important differences in immunohematopoiesis between neuroendocrine hormone-deficient dwarf mice and lethally irradiated and reconstituting mice. Dwarf mice have low circulating levels of pituitary hormones because of the lack of acidophilic anterior cells, but lethally irradiated mice do produce these hormones; therefore, we believe multiple pituitary hormones may influence hematopoiesis. In addition to effects on thymus, we also found that PRL exerted effects on B-cell development and function *in vivo* after BMT. It is also important to note that the hematopoietic growth-promoting effects of rhPRL administration after SBMT occurred at a dose and schedule that did not result in toxicity or significant weight gain. It is known that differentiation and development of various blood cell lineages from hematopoietic progenitor cells and coordination of host immune response are regulated by a group of proteins known as cytokines. Here, we found that prolactin improved the engraftment and reconstitution of human immune cells in SCID mice. Because prolactin may improve the recovery of T cells, B cells and NK cells as well as all myeloid cells, prolactin may be considered as a complementary regimen to treat bone marrow failure and immune deficiency, both of which are critical obstacles in clinical practice. Currently, BMT and cellular adaptive immunotherapy are considered as the promising combined cellular biotherapy regimen, but how to develop the potential of those cells is a critical question to be answered. Because prolactin may improve hematopoietic reconstitution and lymphoid cell recovery in human/mouse chimeras, prolactin will possibly be a potential biological product against cancer in clinical settings. The mechanism by which rhPRL promotes hematopoietic and immune reconstitution after SBMT is not yet clear. The stimulatory effects on hematopoietic progenitor cells may be due to its ability to exert either direct proliferative effects on the progenitor cells or indirect effects by improving the responsiveness of the marrow cells to growth promoting reagents. There is mixed evidence from *in vitro* experiments to support the suggestion that PRL acts directly on hematopoietic progenitor via PRL receptors. It is unclear that the effects of PRL on immunologic and hematopoietic development are direct or indirect.

Prolactin has been postulated to have significant effects on the immune system, but more work needs to be performed to determine the effect of rhPRL on long-term immune reconstitution after SBMT. When ABMT is used in the treatment of cancer, recurrence of the underlying malignancy remains a major cause of treatment failure. Therefore, caution must be taken if using rhPRL to promote hematopoietic recovery in treating a cancer patient with ASBMT, when ABMT is used for the treatment of cancer because of potential effects of rhPRL on tumor growth (49). However, the present report suggests that the pleiotropic actions of rhPRL on various stages and lineages of hematopoietic cell growth after systematic administration may be of considerable use to promote hematopoietic recovery after myelosuppressive therapy, and to stimulate the function of immune competent cell as adjuvant immunotherapy.

We report here that rhPRL can promote human lymphocytes engraftment into peripheral lymphoid organs and improve the production of specific DT-Ig, a kind of T cell-dependent vaccine, in this human/mouse model, which is the first report demonstrating rhPRL can promote human B cell response in an *in vivo* setting. These findings confirm and extend previous reports demonstrating the *in vitro* stimulatory effects of PRL on human B cells. Our results also demonstrated that rhPRL provided a means for optimizing human T and B cell engraftment in SCID mice. It is of interest that rhPRL activation allowed for the entry of human cell within the murine thymus. These findings are in agreement with a report describing that mature T cells could traffic to the murine thymus provided that they were activated, indicating that rhPRL may activate human T cell *in vivo*, which may explain why the specific DT-Ig production was promoted by rhPRL treatment. We have previously demonstrated that treatment of SCID mice receiving huPBL with human growth hormone resulted in the appearance of human cells in the SCID thymus and the results presented here demonstrated yet another stimulus that can be used to promote thymic entry of human T cells. Preliminary evidence suggests that, when human prolactin and human IL-2 are given together, even greater thymic localization was noted (data not shown), suggesting that these two treatments may also be of significant use in

examining the trafficking patterns of xenogeneic cells to the thymus.

We recently reported that GH and PRL exerted differential effects on murine T cell development and function in neuroendocrine hormone-deficient dwarf mice, which have been reported to have deficiencies in T-cell development and function. In contrast to the thymopoietic effects of GH, ovine PRL administration resulted in accelerated thymic atrophy in theses mice. Interestingly, PRL administration had no effect on thymic size in normal mice, suggesting that the lack of other hormones in the dwarf mice made it susceptible to the inhibitory effects of PRL. In this research, we found that PRL administration did exert the engraftment- and function-promoting effects on huamn T cells and B cells. In addition to effects of rhPRL on human, we also found that rhPRL exerted effects on murine B-cell development and function, which have not been clearly reported in the past, demonstrating that rhPRL improved the murine primary Ig response to KLH in syngeneic BM transplanted mice (Zhi-Gang et al. manuscript in preparation), further suggesting that rhPRL may act as a strong adjuvant in human vaccine immunization.

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