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Research Paper

Modeling Human Leukemia Immunotherapy in Humanized Mice

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ABSTRACT

The currently available human tumor xenograft models permit modeling of human cancers in vivo, but in immunocompromised hosts. Here we report a humanized mouse (hu-mouse) model made by transplantation of human fetal thymic tissue plus hematopoietic stem cells transduced with a leukemia-associated fusion gene *MLL-AF9*. In addition to normal human lymphohematopoietic reconstitution as seen in non-leukemic hu-mice, these hu-mice showed spontaneous development of B-cell acute lymphoblastic leukemia (B-ALL), which was transplantable to secondary recipients with an autologous human immune system. Using this model, we show that lymphopenia markedly improves the antitumor efficacy of recipient leukocyte infusion (RLI), a GVHD-free immunotherapy that induces antitumor responses in association with rejection of donor chimerism in mixed allogeneic chimeras. Our data demonstrate the potential of this leukemic hu-mouse model in modeling leukemia immunotherapy, and suggest that RLI may offer a safe treatment option for leukemia patients with severe lymphopenia.

1. Introduction

Mouse models are the most commonly used in vivo systems for cancer studies, in which immunotherapy can be studied in immunologically intact syngeneic hosts. However, because of the many differences between rodents and humans, much of the information on both pathogenesis and immunotherapies learned from the conventional mouse models cannot be applied to humans. For this reason, xenograft models have been increasingly used to study human tumors. In this model, human tumors can be grafted and grow in immunodeficient mice that lack immune system. To study interaction between the tumor and immune system, human peripheral blood mononuclear cells (PBMCs) or T cells were transferred into the immunodeficient mice bearing human tumors. However, the immune responses are not physiological for two reasons. First, xenoantigen-reactive T cells in the injected human cells cause graft-vs.-host responses. Second, because human antigen-presenting cells (APCs) are lacking, such system can neither maintain peripheral T cell pool nor initiate human leukocyte antigen (HLA)-restricted immune responses.

Functional human hematopoietic and lymphoid systems can be established in mice by transplantation of human fetal thymic tissue (FTHY) and $CD34^+$ fetal liver cells (FLCs) to sublethally-irradiated immunodeficient mice (Lan et al., 2006; Lan et al., 2004; Melkus et al., 2006). These hu-mice have proved to be an excellent model for assessing human innate and adaptive immune responses in vivo under normal or pathological conditions such as human immune responses to allo- and xeno-antigens and viral infection (Hu and Yang, 2012). Here, we seek to develop hu-mice with human lymphohematopoietic systems and autologous leukemia that permit exploration of human cancer immunotherapy.

2. Materials and Methods

2.1. Animals and Human Tissues and Cells

NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (NOD/SCID/$\gamma c^{-/-}$ or NSG) mice were purchased from the Jackson Laboratory (Bar Harbor, ME), and were housed in a specific pathogen-free micro-isolator environment and used in experiments at 6 to 10 weeks of age. Human fetal thymus and liver tissues of gestational age of 17 to 20 weeks were obtained from Advanced Bioscience Resource (Alameda, CA). Thymic tissue was cut into small fragments measuring about 1 mm^3; human $CD34^+$ fetal liver cells (FLCs) were purified by a magnetic-activated cell sorter

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(MACS) using anti-human CD34 microbeads (Miltenyi Biotech, Aubum, CA). The prepared human thymic tissue fragments and $CD34^+$ FLCs were then cryopreserved in liquid nitrogen until use. Protocols involving the use of human tissues and animals were approved by the Human Research Committee and the Institutional Animal Care and Use Committee of Columbia University.

2.2. Retroviral Transduction of $CD34^+$ FLCs

Pseudo-typed retroviruses were produced by transfection using Lipofectamine 2000 (Invitrogen, San Diego, CA) of 293FT cells with a 3-plasmid system consisting of the MLL fusion vector (MSCV-*MLL-AF9*-human pgk-*EGFP*; kindly provided by Dr. John Dick) (Barabe et al., 2007) and two packaging vectors (VSV-G and Psi-Env). Viral particles were collected 48 or 72 h post-transfection and concentrated by ultracentrifugation at 50,000 *g* for 2 h, and were titered and stored at −80 °C until use. For transduction, human $CD34^+$ FLCs were stimulated overnight in medium containing 50 ng/mL rhSCF (R&D, Minneapolis, MN), 50 ng/mL Flt-3L (eBioscience, San Diego, CA), 25 ng/mL TPO (R&D, Minneapolis, MN), 10 ng/mL IL-3 (R&D, Minneapolis, MN), in 24-well plates precoated with Retronectin (Takara Bio Inc.), followed by incubation with retroviruses for 12 h. Cells were washed twice and injected into sublethally irradiated NSG mice to generate leukemic hu-mice (see Hu-mouse preparation below). A small aliquot of the transduced cells was cultured for 3 additional days to determine the transduction efficiency by measuring the ratio of GFP^+ cells using FACS (ranged between 10 and 30% in the experiments presented).

2.3. Humanized Mouse Preparation

NSG mice were conditioned with sublethal (2 Gy) total body irradiation (TBI), and transplanted intravenously (i.v.) or intrafemorally (i.f.) with human $CD34^+$ FLCs ($0.5–2 \times 10^5$/mouse) alone or along with thymic tissue fragment measuring about 1 mm^3 (under mouse kidney capsule) from the same fetal donor, as previously described (Lan et al., 2006; Tonomura et al., 2008). Levels of human hematopoietic cells in hu-mice were determined by flow cytometric analysis using various combinations of the following mAbs: anti-human CD45, CD3, CD4, CD8, CD45RA, CD45RO, CD19, CD20, CD10, IgM, IgD, CD44, CD33, CD14, CD15, CD11b, CD11c, CD56, CD34, HLA-DR, HLA-A/B/C; anti-mouse CD45 and Ter119; and isotype control mAbs (all antibodies were purchased from BD PharMingen, San Diego, CA). Analysis was performed on a LSR II (Becton Dickinson, Mountain View, CA), and dead cells were excluded from the analysis by gating out lower forward scatter and high propidium iodide or DAPI-retaining cells. For making hu-mice with autologous leukemia, NSG mice were injected with $CD34^+$ FLCs that were transduced with retroviral vectors containing *MLL*-AF9, and leukemia development was assessed by FACS and histology.

2.4. Cytospin and Histology Analysis

Bone marrow or spleen cells were prepared from leukemic hu-mice and GFP^+ human leukemia cells were purified by cell sorting, suspended in PBS, and centrifuged (130 *g* for 5 min) onto glass slides using a Cytospin centrifuge (Shandon). The slides were stained with the DipQuick Stain Kit (modified Wright Giemsa staining) from Jorgensen Laboratories. Tissues from leukemic hu-mice were fixed in 10% buffered formalin and embedded in paraffin for hematoxylin and eosin (H&E) staining. Stained slides were examined under a Zeiss microscope and photographed using a Nikon Coolpix 5000 digital color camera.

2.5. Hydrodynamic Gene Delivery

Human cytokine genes (IL-15/Flt-3L/GM-CSF/IL-3) were cloned separately into pcDNA3.1(+) vector (Invitrogen) (Chen et al., 2012; Chen et al., 2009). Plasmid DNA was purified by Maxi-prep Kit (Qiagen), and injected i.v. into hu-mice 12 days prior to RLI (5–50 μg of each plasmid in a total of 1.8-mL saline within 7 s using a 27-gauge needle) (Suda et al., 2007).

2.6. In Vivo Human T Cell Depletion

Hu-mice were treated with 6 injections (i.v.) of anti-huCD3-immunotoxin (a gift from Dr. David Neville (Woo et al., 2010)) with the dose of 5 μg/Kg BID for 3 days (6, 5 and 4 days before RLI). Right before each day injections, blood samples were collected for FACS analysis. Some hu-mice were sacrificed to confirm the depletion of human T cells in periphery and organs by FACS 3 days after the treatment was completed.

2.7. Recipient Lymphocyte Infusions

Spleen cells were prepared from RLI-cell source hu-mice and administered i.v. at a dose of $2–3 \times 10^7$ cells per mouse into hu-mouse chimeras 11–12 weeks after human $CD34^+$ cell transplantation. In some experiments, human $CD25^+$ cells were depleted from RLI inoculum by MACS using anti-human CD25 microbeads (Miltenyi Biotech, Aubum, CA).

2.8. Statistical Analysis

The level of significant differences in group means was determined by the Student's *t*-test for parametric data sets. The overall difference between groups was determined by two-way ANOVA with repeated measures. All analysis was performed using Prism 5 (GraphPad Software). A P value of ≤ 0.05 was considered significant in all analyses.

3. Results

3.1. Construction of Humanized Mice With Human Immune System and Autologous Leukemia

We transplanted sublethally-irradiated NSG mice with human FTHY and $CD34^+$ FLCs that were transduced with retrovirus containing a mixed-lineage leukemia (MLL) fusion gene *MLL-AF9* (Barabe et al., 2007) (Fig. 1a). FACS analysis revealed a gradual increase in the levels of human PBMCs, including T and B cells and myeloid cells (or APCs), with a similar kinetics as that seen in hu-mice receiving untransduced $CD34^+$ FLCs (Lan et al., 2006), until 15 weeks when overt leukemia appeared (Fig. 1b). The hu-mice became moribund between 19 and 24 weeks after transplantation (Fig. 1c); autopsy revealed splenomegaly, enlarged lymph nodes, hepatomegaly, and enlarged FTHY grafts in all of these mice (Fig. 1d and data not shown). Histology demonstrated that leukemic cells infiltrated all organs/tissues examined, including bone marrow, spleen, lung, liver, kidney, and FTHY graft (Fig. 2a and data not shown). As Wright-Giemsa staining demonstrated, purified GFP^+ cells exhibited a high nucleus/cytoplasm ratio (Fig. 2b), a typical leukemic blast morphology. The GFP^+ leukemic cells present a B-ALL phenotype, i.e., $CD19^+CD10^+CD20^-$-$sIgM^{low/-}sIgD^{low/-}CD44^{hi}MHC\text{-}I^+MHC\text{-}II^{hi}$ and negative for other lineage markers i.e., $CD33^-CD15^{low/-}CD14^-CD11b^-CD3^-CD4^-$-$CD8^-CD56^-$ (Fig. 2c). Leukemia with a similar B-ALL phenotype also developed in mice receiving *MLL-AF9*-transduced $CD34^+$ FLCs without FTHY (Table 1). Therefore, this leukemic hu-mouse model not only establishes human lymphohematopoietic system, but also