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Keratinocyte Growth Factor and Stem Cell Factor to Improve Thymopoiesis after Autologous CD34$^+$ Cell Transplantation in Rhesus Macaques

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Deficient thymopoiesis and retarded recovery of naive CD4$^+$ T cells are important determinants of insufficient immune-competence following hematopoietic stem cell transplantation (HSCT). Although keratinocyte growth factor (KGF) may protect the thymic epithelium, stem cell factor (SCF) is involved in early thymopoiesis. We evaluated whether KGF alone or combined with SCF would affect thymopoiesis and hematologic recovery following myeloablative autologous HSCT into rhesus macaques. Purpose-bred adult rhesus macaques received 10^6 autologous CD34$^+$-selected mononuclear bone marrow cells (BMC)/kg after 9 Gy myeloablative conditioning. Animals were treated with phosphate-buffered saline (PBS) (n = 2), KGF alone (n = 2), or KGF combined with SCF (n = 2). KGF-treated animals showed accelerated hematologic recovery, improved thymopoiesis, and enhanced naive T-cell recovery following transplantation. Improved T cell recovery was not associated with protection against cytomegalovirus reactivation nor with improved antibody response to tetanus toxoid vaccination. Animals treated with KGF and SCF experienced severe adverse events that precluded evaluation of thymopoiesis and T cell recovery. Collectively, our data confirm that KGF may enhance thymopoiesis.

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INTRODUCTION

Protracted recovery of naive CD4$^+$ T cells in recipients of allogeneic hematopoietic stem cell transplantation may result in a prolonged susceptibility to life-threatening opportunistic infections [1-3]. Reconstitution of naive CD4$^+$ T cells depends on the differentiation of stem cell-derived lymphoid progenitor cells into mature, naive CD4$^+$ T cells in the thymus (ie, thymopoiesis). Recovery of thymopoiesis has been shown to be pivotal for the generation of a new diverse T cell receptor repertoire and long-term immunity [4-6]. High-dose chemo- and radiotherapy, aging and graft-versus-host disease (GVHD) may adversely affect both the thymic stromal compartment and developing thymocytes, resulting in impaired CD4$^+$ T cell recovery following allogeneic hematopoietic stem cell transplantation (HSCT) [7-9]. Strategies to protect the thymic stroma or to stimulate thymocyte development may improve the recovery of naive T cells and restore the repertoire to combat a variety of infectious microorganisms [10-12]. Keratinocyte growth factor (KGF) is a growth factor that protects thymic stroma, whereas stem cell factor (SCF) stimulates outgrowth of T cell progenitors.

KGF was initially discovered as a stimulator of epithelial cell growth and is produced by cells of mesenchymal origin. Its receptor is expressed by epithelial cells including thymic epithelial cells [13,14]. Exogenous KGF experimentally enhances thymopoiesis in normal mice, reverses age-associated thymic involution, and protects thymic epithelial cells against damage caused by irradiation, chemotherapy, and GVHD [15-18]. Thymic regeneration and T-cell reconstitution were enhanced in mice and nonhuman primates treated with KGF before allogeneic or autologous HSCT [14,17,19].

SCF is a cytokine produced by stromal cells including thymic stroma and c-kit; the SCF receptor is expressed by the earliest thymocytes [20-23]. SCF is important for proliferation and differentiation of early thymic T cell progenitors in vitro and in vivo.

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c-kit$^{-/-}$ or SCF$^{-/-}$ mice show a block in thymocyte differentiation [24,25], and SCF$^{-/-}$ thymi poorly support early thymocyte expansion [26]. In addition, differentiation of early thymic progenitors into the T cell lineage is dependent on c-kit signaling and Notch- and IL-7-induced proliferation require c-kit signaling in vitro [27]. Administration of SCF in mice accelerated leukocyte recovery following radiation-induced myeloablation [28] but not following 5-fluorouracil (5-FU) treatment [29] or myeloablative HSCT [30]. Recently, we showed that SCF administration improved thymopoiesis in a humanized mice model [31]. In the present study, we evaluated the effect of KGF alone or combined with SCF on T cell reconstitution and thymopoiesis following myeloablative autologous HSCT into rhesus macaques.

MATERIALS AND METHODS

Animals

Purpose-bred male rhesus monkeys (*Macaca mulatta*), weighing 2.9-3.8 kg and age 2.5-3.7 years, were used. Monkeys were housed in groups of 4 to 6 in stainless steel cages in rooms equipped with a reverse filtered air barrier, normal daylight rhythm, and conditioned to 20°C with a relative humidity of 70%. Animals were fed ad libitum with commercial primate chow and fresh fruits, and received acidified drinking water. All animals were free of intestinal parasites, and seronegative for herpes B, simian T-lymphotropic viruses, and simian immunodeficiency virus. Housing, experiments, and all other conditions were approved by an ethical committee in conformity with legal regulations in the Netherlands. Studies with rhesus monkeys were done sequentially using highly codified methods, including radiation and placebo controls at regular intervals. For the present study, assignment to the study groups was random.

Collection of CD34$^+$ Hematopoietic Progenitor Cells

Bone marrow (BM) was aspirated and low-density cells isolated as previously described and red blood cells reinfused immediately after cell separation [32,33]. CD34$^+$ cells were isolated by immunomagnetic separation using an IgG2a antibody against CD34 (mAb 561 kindly provided by T. Egeland, University of Oslo, Oslo, Norway), which was noncovalently linked to rat-antimouse IgG2a beads (Dynal, Oslo, Norway) [34]. CD34$^+$ cells devoid of the anti-CD34 antibody were recovered using a polyclonal antibody against the Fab part of the anti-CD34 antibody (Detachabead, Dynal). CD34-selected cells (10^6/kg) were reinfused into the monkeys within 24 hours after radiation.

Total Body Irradiation (TBI) and Supportive Care

Rhesus monkeys were irradiated with a single dose of 9 Gy TBI delivered by a 6-MV linear accelerator (Siemens [Siemens Healthcare, Erlangen, GE]) as described [33]. Two weeks before TBI, the monkeys were placed in a laminar flow cabinet, the gastrointestinal tract was selectively decontaminated, and iron ws supplemented as previously described [32,33]. Supportive care after irradiation by infection prevention, transfusions, and maintenance of a hydration state were done as previously described [32,33]. Platelet transfusions had a mean volume of 12.8 ± 3.4 mL and contained 7.0 ± 3.0 × 10^9/L platelets. Whole-blood transfusions consisted of 38.1 ± 10.8 mL, containing 0.17 ± 0.05 × 10^{12}/L erythrocytes and 10.0 ± 3.1 × 10^9/L platelets.

Cytokine Administration

Recombinant human KGF (palifermin, Kepivance®) and recombinant human stem cell factor (SCF) were kindly provided by Amgen (Thousand Oaks, CA). One group of animals (n = 2) received KGF at a dose of 200 μg/kg body weight by intravenous bolus at days −3, −2, −1, 0, +1, and +2. A second group of animals (n = 2) received both KGF and SCF. SCF was administered at a dose of 200 μg/kg body weight by subcutaneous injection daily from day 5 until day 45 posttransplantation and KGF as outlined above [35,36]. A third group of animals (n = 2) was used as control group and received phosphate-buffered saline (PBS) injections (Figure 1).

Collection of Peripheral Blood and Tissue Samples

Complete blood cell counts were measured daily using the ABC-vet animal blood counter (Scil, ABX diagnostics, Montpellier, France). Peripheral blood and BM samples for flow cytometry and molecular analysis were drawn once weekly. Animals were euthanized between day 190 and 212 following transplantation, and lymphoid tissues were collected and either processed into a single-cell suspension or fixed in 10% formalin and paraffin-embedded for light microscopic analysis. Thymic architecture was graded using a previously described grading system [19].

Flow Cytometric Analysis

At weekly intervals, absolute numbers of peripheral blood leukocyte subsets were determined by single platform flow cytometry as described previously for murine experiments [37]. mAbs against rhesus macaque epitopes used for flow cytometric analysis were anti-CD45 (D058-1283), anti-CD3 (SP34-2), anti-CD4 (L200), anti-CD8 (SK1/2ST8.5H7), anti-CD20 (L27), anti-CD16/56 (3G8/MY31), anti-CD14 (M5E2), anti-CD28 (CD28.2/L293), anti-CD95 (Dx2),