Immunobiology 219 (2014) 131–137

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

Long term human reconstitution and immune aging in NOD-Rag (−)-γ chain (−) mice

David T. Harris*, Michael Badowski

Department of Immunobiology, University of Arizona, Tucson, AZ 85724, United States

ARTICLE INFO

Article history:
Received 29 June 2013
Received in revised form 20 August 2013
Accepted 26 August 2013
Available online 5 September 2013

Keywords:
Cord blood
CD34
Stem cells
NRG mice

ABSTRACT

Aging of the human immune system is characterized by a gradual loss of immune function and a skewing of hematopoiesis toward the myeloid lineage, a reduction in the lymphocytic lineage, and progressive increases in senescent memory T cells at the expense of naïve T cells. Both the innate and the adaptive branches of the immune system are affected, including neutrophils, macrophages, dendritic cells and lymphocytes. Mice, the most common research model, although inexpensive, do not necessarily reflect the human immune system in terms of its interaction with infectious agents of human origin or environmental factors. This study analyzed whether a human immune system contained within the NOD-Rag (−)-γ chain (−) mouse model could realistically be used to evaluate the development and therapy of aging-related diseases. To that end lightly irradiated NOD-Rag (−)-γ chain (−) mice were injected intrahepatically on day 1 of life with purified cord blood-derived $CD34^+$ stem and progenitor cells. Multiple mice were constructed from each cord blood donor. Mice were analyzed quarterly for age-related changes in the hematopoietic and immune systems, and followed for periods up to 18–24 months post-transplant. Flow cytometric analyses were performed for hematopoietic and immune reconstitution. It was observed that NOD-Rag (−)-γ chain (−) mice could be "humanized" long-term using cord blood stem cells, and that some evidence of immune aging occurred during the life of the mice.

Introduction

Aging of the human immune system is characterized by a gradual loss of immune function and a skewing of the hematopoietic cells toward the myeloid (CD14) lineage, with a reduction in the lymphocytic lineage (T, B and NK cells), and progressive increases in senescent memory T cells at the expense of naïve T cells ($CD3^+45RO^+28^-$; Dorshkind et al. 2009; Gomez et al. 2005; Maue et al. 2009). Both the innate (Gomez et al. 2005) and the adaptive (Dorshkind et al. 2009) branches of the immune system are affected, including neutrophils (PMN), macrophages, dendritic cells (DC) and lymphocytes. The hematopoietic stem cell (HSC) population is also detrimentally affected by aging as reflected by its inability to maintain both hematopoiesis and lymphopoiesis (Rossi et al. 2005). Mice, the most common research model, although inexpensive, do not necessarily reflect the human immune system in terms of its interaction with infectious agents of human origin or environmental factors. Therefore, a major challenge for immunology and gerontology is to develop a model that functionally mimics human immune system development, function and senescence, which is relatively inexpensive, and can be manipulated to explore the intricacies of aging. Thus there is a need for a humanized animal model in which triggers of immune aging and treatment variables can be manipulated in order to delineate optimal therapeutic regimes. The model analyzed in this study built upon work reported in the literature developing humanized mice (Traggiai et al. 2004; Brehm et al. 2010; Shultz et al. 2010), and expanded these studies to include aged humanized animals.

In aging there is a progressive decline in tissue and organ function along with a loss of stem cell numbers and/or function (Rossi et al. 2005; Hattiangady and Shettya 2008; Zhou et al. 2008; Yu et al., 2011; Sowa et al. 2012). As individuals age there is an increased incidence of myeloid cancers but it is unknown if any of these age-related changes are due to intrinsic or extrinsic factors. Although polycomb group proteins are more likely to become methylated with age it is unclear if this observation is a cause or effect of aging (Teschendorff et al. 2010). However, recent work (Mayak et al. 2010; Conboy et al. 2005) has shown that HSC aging can be reversed by exposure to a younger environment implying that non-cell intrinsic local and systemic factors are involved, which may be evolutionarily conserved (e.g. IGF-1 and Notch signals). Similar "rejuvenation" results have been seen with muscle as well (Conboy

Abbreviations: CB, cord blood; NRG, NOD/$RAG^{-/-}$/$IL2R\gamma^{null}$; PMN, polymorphonuclear cells; HSC, hematopoietic stem cells.

* Corresponding author at: Department of Immunobiology, University of Arizona, PO Box 245221, Tucson, AZ 85724, United States. Tel.: +1 520 626 5127; fax: +1 520 626 2100.
E-mail address: davidh@email.arizona.edu (D.T. Harris).

0171-2985/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.imbio.2013.08.013

et al. 2003). It is currently unknown if a similar result would be obtained with the innate and adaptive immune systems, both of which decline with age. If so, aging might best be treated by targeting the extrinsic environmental factors rather than cell intrinsic factors (Kovacs et al. 2009).

Over the past four years a number of novel humanized mouse strains have been developed in an attempt to provide model systems to address some of these issues, albeit there is some debate as to whether one model is better than another. Many of these models have utilized purified umbilical cord blood CD34$^+$ stem cells injected into newborn (triply) immunodeficient mice which then reconstitute the animal with some or all parts of the human innate and adaptive immune systems. There have been questions as to whether a truly functional (as opposed to phenotypic) human immune system develops, whether it represents a diverse and properly restricted adaptive immune system (focused on recognition of human HLA molecules), and if all parts of the innate immune system are intact. Data has been published to support and refute each of these claims (Traggiai et al. 2004; Brehm et al. 2010; Shultz et al. 2010; Liu et al. 2010; Watanabe et al. 2009; Melkus et al. 2006; Marodon et al. 2009; Freitas et al. 1989; Strowig et al. 2010; Chang et al. 2012). Essentially no work has been published with regard to the use of these systems to study aging and its effects on the human hematopoietic and immune systems (Maue et al. 2009; Weng et al. 2009).

The intent of the current study was to determine the effects of host aging on the humanized blood and immune system contained within NOD-Rag1^{null}-γ chain^{null} (NRG) mice constructed from a single stem cell donor, as measured by flow cytometric analyses. We serially sampled the same mice over a period of time of 1 year or longer. It was observed that NRG mice could be "humanized" long-term using cord blood stem cells, and that such animals do exhibit some signs of immune system aging.

Materials and methods

Mice

Humanized mice were created using a variation of the NOD-Rag1^{null}-γ chain^{null} (NRG) model as described in the literature (Chicha et al. 2005). Briefly, female mice homozygous for both the Rag1^{null} and IL2rγ^{null} (common gamma chain null) mutations were bred with male mice homozygous for the Rag1 knockout mutation and hemizygous for the X-linked IL2rγ^{null} mutation, as obtained from Jackson Labs (Bar Harbor, ME). Mice were bred at the University of Arizona animal facilities, an AAALAC-approved institution.

Cord blood collections and CD34 purifications

Cord blood (CB) samples were collected, processed and cryopreserved as previously described (Harris et al. 1994) under an IRB-approved protocol. CD34$^+$ CB stem cells were enriched using magnetic beads columns (StemCell Technologies, Vancouver, Canada), as per the manufacturer's instructions, to 95% or greater purity.

Transplantation

Newborn mice were irradiated in a 3–4 h interval (2 Gy × 2 Gy) using a cesium or cobalt source. At 4–12 h post-radiation, mice were transplanted intra-hepatically with CD34$^+$ (>95% purity) human cord blood stem cells (30 μl in PBS, 2–4 × 10^5 cells/mouse) collected from healthy, disease-free mothers and infants. The mice were anesthetized, the liver was visualized through the skin, and cord blood stem cells are directly injected into the organ. This procedure was performed by the EMSS (Experimental Mouse Shared Service) of the Arizona Cancer Center at the University of Arizona. Mononuclear cells from the cord blood collection used for transplantation were frozen and stored for later use in future experiments. Engraftment of human cells was ascertained by flow cytometric (FACS) analyses of peripheral blood obtained from each transplanted mouse.

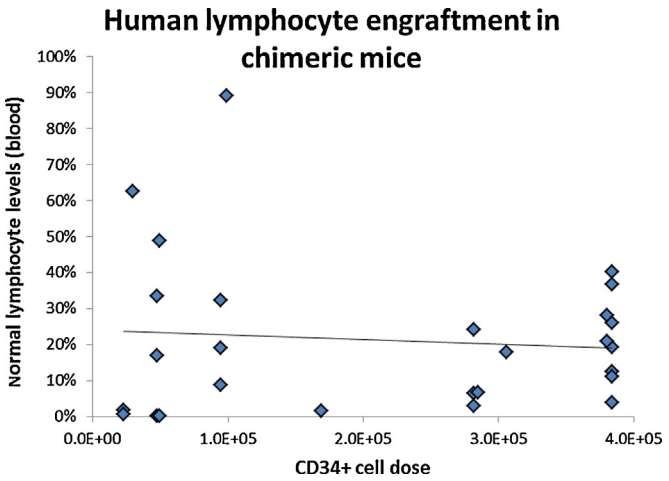

Fig. 1. Engraftment *versus* CD34$^+$ cell dose. Newborn NRG mice were irradiated and injected intra-hepatically with the indicated dose of purified CD34$^+$ cord blood stem cells (*x*-axis) as described in "Materials and methods" section. Engraftment was determined at days 90–120 by flow cytometry for the presence of human T, B and myeloid cells. Total human leukocyte cell counts were determined and compared to normal murine blood leukocyte cell counts (*y*-axis). Each symbol represents an individual mouse. The trend line indicated no significant correlation of cell dose with engraftment.

FACS analysis

The humanized mice were characterized 90–120 days post-transplant by the presence of human immune cells as determined by flow cytometry using heparinized blood obtained from the submandibular pouch (i.e., presence of CD45$^+$, CD14$^+$, CD3$^+$, CD19$^+$, CD16$^+$, CD11c$^+$ populations) of each animal. Samples were analyzed either with a FACScan flow cytometer (BD, Franklin Lakes, NJ), with single laser emitting at 488 nm, a LSRII using lasers emitting at 633 nm and 532 nm. Data was analyzed and displayed with FacsDiva software or Cytlogic. A minimum of 20,000 gated events were analyzed for each sample. Antibodies were obtained from Biolegend (San Diego, CA), BD (Franklin Lakes, NJ) and Caltag Laboratories (Burlingame, CA). Gating was performed on viable cells as based on forward and side scatter parameters. Human cells were identified based on anti-human CD45 mAb binding. In addition comparable anti-mouse leukocyte mAbs were added to each tube to identify any possible contribution of mouse leukocytes to overall mAb binding. Red blood cells were lysed using standard Tris–ammonium chloride buffer prior to staining and analysis.

Results

Long term engraftment and survival parameters

NRG mice were transplanted with CB CD34$^+$ cells on d1 of life as described in "Materials and methods" section. At 90–120 days post-transplant mice were analyzed for the presence of human cells in the peripheral blood. As shown in Fig. 1 the level of human cell engraftment varied from less than 10% of normal murine blood levels to greater than 90%, with the average engraftment being approximately 30% of expected normal blood cell numbers. It was