Blood Cells, Molecules and Diseases 55 (2015) 15–20

Contents lists available at ScienceDirect

Blood Cells, Molecules and Diseases

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

Ames hypopituitary dwarf mice demonstrate imbalanced myelopoiesis between bone marrow and spleen

Maegan L. Capitano [a], Brahmananda R. Chitteti [b], Scott Cooper [a], Edward F. Srour [b], Andrzej Bartke [c], Hal E. Broxmeyer [a,*]

[a] Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN, USA
[b] Department of Medicine, Indiana University School of Medicine, Indianapolis, IN, USA
[c] Department of Internal Medicine, Geriatrics Research, Southern Illinois University School of Medicine, Springfield, IL, USA

ARTICLE INFO

Article history:
Submitted 26 March 2015
Accepted 26 March 2015
Available online 28 March 2015

Keywords:
Ames dwarf mice
Hematopoietic progenitor cell
Growth hormone
Prolactin
Thyroid-stimulating hormone

ABSTRACT

Ames hypopituitary dwarf mice are deficient in growth hormone, thyroid-stimulating hormone, and prolactin. The phenotype of these mice demonstrates irregularities in the immune system with skewing of the normal cytokine milieu towards a more anti-inflammatory environment. However, the hematopoietic stem and progenitor cell composition of the bone marrow (BM) and spleen in Ames dwarf mice has not been well characterized. We found that there was a significant decrease in overall cell count when comparing the BM and spleen of 4–5 month old dwarf mice to their littermate controls. Upon adjusting counts to differences in body weight between the dwarf and control mice, the number of granulocyte-macrophage progenitors, confirmed by immunophenotyping and colony-formation assay was increased in the BM. In contrast, the numbers of all myeloid progenitor populations in the spleen were greatly reduced, as confirmed by colony-formation assays. This suggests that there is a shift of myelopoiesis from the spleen to the BM of Ames dwarf mice; however, this shift does not appear to involve erythropoiesis. The reasons for this unusual shift in spleen to marrow hematopoiesis in Ames dwarf mice are yet to be determined but may relate to the decreased hormone levels in these mice.
© 2015 Elsevier Inc. All rights reserved.

1. Introduction

The pituitary gland secretes several hormones including: growth hormone (GH), prolactin (PRL) and thyroid-stimulating hormone (TSH). These hormones are secreted by three specific adenohypophyseal cell types: somatotrophs which produce GH in response to GH-releasing hormone, lactotrophs which produce PRL in response to estrogen, progesterone, and thyrotropin-releasing hormone, and thyrotrophs which produce TSH in response to thyrotropin-releasing hormone. These hormones are essential for growth, maintaining blood pressure, some aspects of pregnancy, child birth, and nursing, kidney function, pain relief, regulating body temperature and the function of sex organs and thyroid glands. The Ames dwarf mice are used to study the role of hypopituitarism on various biological functions. Ames dwarf mice are homozygous for a recessive loss of function mutation at the Prop1 locus ($Prop1^{df/df}$) [1–3]. The loss of function mutation at Prop1 interferes with development of the adenohypophyseal cell types, thus leading to deficiencies in GH, PRL and TSH. These hormone deficiencies also lead to suppressed circulating levels of insulin-like growth factor 1 (IGF-1), thyroid hormones (e.g., thyroxin and triiodothyronine), insulin and glucose [1,4]. Therefore, Ames dwarf mice demonstrate increased insulin sensitivity, glucose tolerance and hypothyroidism.

This significant alteration to the physiology of hypopituitary mice leads to a very unique phenotype. First, $Prop1^{df/df}$ mice are dwarfs that demonstrate a significantly longer lifespan than $Prop1^{+/+}$ or $Prop1^{+/df}$ littermates with an ~50% longer lifespan in males and a >60% longer lifespan in females [4,5]. Second, they have delayed occurrences of fatal neoplastic diseases suggesting that these mice do age like normal mice, but differences in the aging phenotype do not occur until the mice are much older [5,6]. Finally, hypopituitary mice demonstrate a significant reduction in the number of bone marrow cells, splenocytes

Abbreviations: GH, growth hormone; PRL, prolactin; TSH, thyroid-stimulating hormone; IGF-1, insulin-like growth factor-1; TNFα, tumor necrosis factor alpha; IL, interleukin; HSC, hematopoietic stem cell; HPC, hematopoietic progenitor cell; NOD, non-obese diabetic; SCID, severe combined immunodeficiency; LT-HSC, long term-hematopoietic stem cell; ST-HSC, short term-hematopoietic stem cell; MPP, multipotent progenitors; CMP, common myeloid progenitors; GMP, granulocyte-macrophage progenitors; MEP, megakaryocyte–erythrocyte progenitors; CLP, common lymphoid progenitors; rmGM-CSF, recombinant mouse granulocyte macrophage colony-stimulating factor; rmM-CSF, recombinant mouse macrophage colony-stimulating factor; rmIL-3, recombinant mouse interleukin-3; rmSCF, recombinant mouse stem cell factor; rhuEPO, recombinant human erythropoietin; CFU-GM, granulocyte-macrophage colony-forming units; BFU-E, erythrocyte burst-forming unit; CFU-GEMM, granulocyte, erythrocyte, monocyte, megakaryocyte CFU.

* Corresponding author at: Department of Microbiology and Immunology, Indiana University School of Medicine, R2 Room 302, 950 West Walnut Street, Indianapolis, IN 46202, USA.
E-mail address: hbroxmey@iupui.edu (H.E. Broxmeyer).

http://dx.doi.org/10.1016/j.bcmd.2015.03.004
1079-9796/© 2015 Elsevier Inc. All rights reserved.

and thymocytes even after adjusting for differences in body weight between dwarf mice and their littermate controls [7–12].

B cell development in the bone marrow of ~1–4 month old hypopituitary mice is defective as indicated by a decrease in pre-, pro- and total B cell numbers when compared to littermate controls [9,12,13]. T cell development is also deficient in these mice as indicated by a reduction in double positive ($CD4^+CD8^+$) T cells in the thymus and the abnormal presence of double positive T cells in the lymph nodes [9,12,13]. In addition to abnormal T and B cell development, functional studies have shown that both cell-mediated and humoral immunity is compromised in hypopituitary mice [7,10,11]. Ames dwarf mice have an overall reduction in the ability of T cells to respond to super antigen compared to their littermate controls. Ectopic pituitary transplants restored Ames dwarf mice to immunocompetence by enhancing the number of lymphocytes and their natural killer activity suggesting that restoring PRL levels alters the immunodeficiency seen in Ames dwarf mice [14]. These findings are also observed in other pituitary dwarf syndromes such as the Snell–Bagg/Snell dwarf mice and in mice and humans in which GH, PRL, thyroxine, and IGF-1 functions were impaired [8,11, 13–15]. However, it is important to note that in the Snell–Bagg dwarf mice, the differences seen in the immune system are dependent on when the mice were weaned [16].

A microarray analysis of 34,000 genes in the peripheral blood leukocytes from 7 month old Ames dwarf mice identified 6 main genes which had altered expression in Ames dwarf mice compared to littermate controls: casp3, bcl2, IL4, mapk14, TGFβ1 and pcrk [17]. The function of these genes suggests that Ames dwarf mice may have functional changes in apoptosis, B and T cell homeostasis, prostaglandin synthesis, humoral immunity, chemokine activity, complement activation, and wound healing suggesting that Ames dwarf mice have several anti-inflammatory pathways activated. This is further supported by the fact that Ames dwarf mice have increased levels of adiponectin that can act as an anti-inflammatory factor [18–20]. GH regulates the level of adiponectin produced by adipose tissues and in the absence of GH signaling adipose tissue increases its release of adiponectin and reduces the secretion of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNFα) and interleukin (IL)-6 [21]. Interestingly, disruption of GH receptor in adipose tissue alone results in normal adiponectin levels unlike what occurs in global GH receptor knockout mice suggesting that GH indirectly regulates adiponectin levels [22].

Although it has been fairly well established that pituitary hormonal deficiencies lead to abnormalities in the immune response, little is known of what is occurring to the hematopoietic stem cell (HSC) and the hematopoietic progenitor cell (HPC) populations in the bone marrow and spleen of hypopituitary mice. It has been previously published that there was an increase in a $Lin^-Sca1^+CD45^+$ population and a trend increase in clonogeneic progenitor cells in the bone marrow of Ames dwarf mice when compared to littermate controls [23]. In this paper, through the utilization of a more extensive panel of markers for immunophenotyping and by colony-formation assays, we examined HSC and HPC composition of the bone marrow and spleen in greater detail to elucidate alterations in hypopituitary Ames dwarf mice.

2. Materials and methods

2.1. Mice

Non-obese diabetic (NOD)/severe combined immunodeficiency (SCID) mice (8–10 week old females) were obtained from an on-site breeding core facility at Indiana University School of Medicine. Male and female Ames dwarf ($Prop1^{df}$) homozygous (df/df) and littermate control (+/df) mice were bred at Southern Illinois University then transferred to Indiana University School of Medicine. Details of the animal husbandry were described previously [24]. Briefly, Ames df/df mice were produced by mating heterozygous females and homozygous mutant males. All breeding protocols were approved by the Southern Illinois University Laboratory Animal Care Committee. All further animal procedures were approved by the Indiana University Committee on Use and Care of Animals. Animals were maintained under temperature-and light-controlled conditions (21–24 °C, 12 hour light/12 hour dark cycle). Animals were group-housed according to age, sex and genotype. Mice were fed ad libitum. Mice were between 4–5 months of age at time of use. Immediately following euthanization, mice were weighed utilizing a Mettler PM2000 scale (Mettler Toledo; Columbus, OH) and femurs and spleens excised.

2.2. Flow cytometry

Immunophenotyping of stem and progenitor cells was performed by flushing femurs of Ames dwarf (df/df) or littermate control (+/df) mice and incubating the bone marrow cells with fluorochrome conjugated anti-mouse antibodies in PBS at room temperature for 25 min. One μg antibody was used per one million cells for each antibody. All antibodies were purchased from either BD Biosciences (San Diego, CA) or BioLegend (San Diego, CA). For bone marrow HSC and HPC analysis the following panel of antibodies were used: FITC-conjugated anti-mouse lineage cocktail (anti-CD3/Gr-1/CD11b/B220/Ter-119; BioLegend), PE-Cy7-conjugated anti-mouse Sca1 (clone D7), APC-H7-conjugated anti-mouse c-Kit (clone 2B8), PE-conjugated anti-mouse CD34 (clone RAM34), APC-conjugated anti-mouse Flk2 (clone A2F10.1), PerCp-Cy5.5-conjugated anti-mouse FcγR (clone 2.4G2) and BV421-conjugated anti-mouse IL-7R (clone SB/199) all purchased from BD Biosciences with their appropriate isotype controls. Long term-hematopoietic stem cells (LT-HSC) were defined as $Lin^-Sca1^+c\text{-}Kit^+CD34^-Flk2^-$. Short term (ST)-HSCs are defined as $Lin^-Sca1^+c\text{-}Kit^+CD34^+Flk2^-$. Multipotent progenitors (MPP) were defined as $Lin^-Sca1^+c\text{-}Kit^+CD34^+Flk2^+$. Common myeloid progenitors (CMP) were defined as $Lin^-Sca1^-c\text{-}Kit^+CD34^{int}Fc\gamma R^{lo}$. Granulocyte-macrophage progenitors (GMP) were defined as $Lin^-Sca1^-c\text{-}Kit^+CD34^{hi}Fc\gamma R^{hi}$. Megakaryocyte–erythrocyte progenitors (MEP) were defined as $Lin^-Sca1^-c\text{-}Kit^+CD34^{lo}Fc\gamma R^{lo}$. Common lymphoid progenitors (CLP) were defined as $Lin^-Sca1^{lo}c\text{-}Kit^{lo}Flk2^+IL\text{-}7R^+$. Data were acquired on an LSRII flow cytometer (BD Biosciences). Single color compensation and isotype controls were included in each experiment. Data analysis was performed using FlowJo 7.6.3 software (TreeStar, WA). Gates were determined using fluorescence minus-one controls. An example of the gating strategy to determine the percent of each population is given in Supplementary Fig. 1. The percent of each population was used to calculate the absolute number of each phenotype of stem and progenitor cells per femur. Once the number of cells per femur was determined the number of cells for the dwarf mouse was adjusted based on the difference in weight using the following formula: [(average weight of littermate control mice/individual dwarf mouse weight)* the number of cells per femur].

2.3. HPC assays

For HPC assays performed in agar, bone marrow cells flushed from the femurs of Ames dwarf (df/df) or littermate control (+/df) mice were plated at 5×10^4 cells/mL in 0.3% semi-solid agar medium with 10% FBS (Fisher Scientific; Waltham, MA) that did or did not contain 10 ng/mL recombinant mouse granulocyte macrophage colony-stimulating factor (rmGM-CSF; R&D Systems; Minneapolis, MN), 10 ng/mL recombinant mouse macrophage colony-stimulating factor (rmM-CSF; R&D Systems), 10 ng/mL recombinant mouse interleukin-3 (rmIL-3; R&D Systems) and 50 ng/mL recombinant mouse stem cell factor (rmSCF; R&D Systems). Colonies were scored after 6 days of incubation at 5% CO_2 and lowered (5%) O_2 in a humidified chamber. For HPC assays performed in methylcellulose, bone marrow cells flushed from femurs or splenocytes isolated from spleens of Ames dwarf (df/df) or littermate control (+/df) mice were plated at 2.5×10^4 (spleen) or

Download English Version:

https://daneshyari.com/en/article/2827066

Download Persian Version:

https://daneshyari.com/article/2827066

Daneshyari.com