

BONE MARROW TRANSPLANTATION: A GENERAL DISCUSSION***¹Professor D. K. Awasthi, ²Dr. Archana Dixit, ³Professor N. K. Awasthi and ⁴Ashutosh Dewedi**¹Department of Chemistry sri JNMPG College Lucknow Uttar Pradesh India.²Department of Chemistry D.G.College Kanpur Uttar Pradesh India.³Department of Chemistry BSNVPG College Lucknow Uttar Pradesh India.⁴Department of Chemistry Resarch Student Sri JNMPG College Lucknow Uttar Pradesh.***Corresponding Author: Professor D. K. Awasthi**

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ABSTRACT

Bone marrow transplantation has evolved over a period of 50 years. Laboratory observations and animal studies defined the essentials of transplantation biology. The first attempts to transfer these studies to patients met with little success. The definition of the complexities of the human leukocyte antigen (HLA) system made it possible to select compatible sibling donors and more recently unrelated donors. Transplantation of stem cells from marrow, blood, or cord blood is now the treatment of choice for a variety of hematological and genetic diseases. Transplantation using less toxic preparative regimens to induce mixed chimerism makes possible an application to autoimmune diseases. Laboratory and clinical research directed toward induction of tolerance and elimination of malignant cells point the way to a wider application of hematopoietic cell transplantation in the next decade.

KEYWORDS: human leukocyte antigen (HLA), chimerism.**INTRODUCTION**

The story of marrow transplantation began in 1949 with the studies of Jacobson et al.^[36] who found that shielding the spleen of a mouse during otherwise lethal irradiation permitted survival. Lorenz et al.^[45] reported that irradiated mice could also be protected by an infusion of spleen or marrow cells. Initially, it was thought that the irradiation protection phenomenon was due to humoral factors. However, in 1954 Barnes and Loutit reviewed their own and other experiments and stated the chemical hypothesis has not been proved by the complete exclusion of the cellular hypothesis.^[3] Strong support for the cellular hypothesis came in 1955 when Main and Prehn^[47] reported that irradiated mice protected by an infusion of allogeneic marrow subsequently displayed tolerance of a donor skin graft. Shortly thereafter, Ford et al.^[26] showed that lethally irradiated mice protected by a subsequent marrow infusion had marrow cytogenetic characteristics of the donor.

In the 1950s, fundamental observations were made in murine systems as detailed in the book by van Bekkum and DeVries.^[95] It was found that allogeneic marrow cells successfully engrafted could mount an immune attack against the host, resulting in a wasting syndrome known as secondary disease. The disease was the result of an immune reaction of the engrafted lymphoid cells against the tissues of the host, now known as graft-

versus-host disease (GVHD).^[6] Uphoff reported that in allogeneic transplants the severity of the immune reaction of donor cells against the host was controlled by genetic factors.^[93] Also, Methotrexate (MTX), as an immunosuppressive agent, could prevent or ameliorate the graft-versus-host (GVH) reaction.^[43,94] In the 1960s, studies in canines provided important information about bone marrow transplantation (BMT) in outbred species applicable to humans. It was found that dogs could survive 2 to 4 times the lethal exposure to total body irradiation (TBI) if given an intravenous infusion of marrow cells set aside or cryopreserved before the TBI.^[49] Dogs given supralethal irradiation and allogeneic marrow demonstrated the problems of failure of engraftment, graft rejection, engraftment with GVHD and, in some dogs, stable engraftment without GVHD, i.e., tolerance.^[86] 211 Bone Marrow Transplantation: A historical review It is now known Dogs could be successfully engrafted without TBI using chemotherapy with cyclophosphamide (CY) or dimethyl busulfan.^[71] Marrow grafts between littermate pairs matched for dog leukocyte antigens (DLA) were often successful with the recipients becoming healthy chimeras.^[22,23] Hematopoietic cells for engraftment could be obtained from the blood as well as the bone marrow.^[15] Blood transfusions from the marrow donor or unrelated dogs could sensitize the intended recipient to transplantation antigens resulting in graft failure.^[72] Proof of the

importance of leukocyte groups in hematopoietic cell transplantation (HCT) came from studies of the DLA system.^[23,75] Dogs given supralethal irradiation and marrow from a DLA mismatched littermate died of graft rejection or GVHD. Most recipients of DLA matched marrow, especially those given some post-grafting MTX to suppress the GVH reaction, became long-term healthy survivors.

THE BEGINNING OF THE MODERN ERA OF BMT:

By the end of the 1960s, platelet transfusion support, improved antibiotics and more effective anticancer agents had been developed. Increasing knowledge of human histocompatibility antigen systems led to renewed attempts at allogeneic marrow grafting in human patients. Gatti *et al.*^[27] reported a successful allogeneic marrow graft in a patient with severe combined immunological deficiency using a sibling donor presumed to be HLA identical with the patient. Subsequent typing, however, showed that the patient and donor differed by one HLA antigen. Two similar successes were reported at almost the same time.^[1,21] The patients did not require immunosuppressive therapy since they were already immune incompetent because of their disease. All three were alive and well 25 years later.^[10] In 1969 the Seattle marrow transplant team began a series of marrow transplantations using HLA matched sibling donors for patients in the end stages of leukemia^[14] or aplastic anemia.^[84] In 1975, a review article summarized the state of knowledge of BMT at that time.^[91] The article described the results in 37 patients with aplastic anemia and 73 with leukemia, all transplanted after failure of conventional therapy. Engraftment was successful in some patients with aplastic anemia and survival with grafts in remission was observed in a few patients with leukemia. In the 1970s evaluation of the role of hematopoietic cell transplantation (HCT) in the treatment of leukemia was difficult because almost all patients had been transplanted for advanced disease after failure of conventional therapy. In 1977 the Seattle team reported 100 patients with advanced acute leukemia who were prepared with CY and TBI and given marrow from an HLA matched sibling.^[81] At the time of the report, 17 of the 100 were alive 1 to 3 years later. Eight of these 17 are alive and well now more than 23 years after transplantation. The early demonstration of a plateau in a Kaplan-Meier plot of disease-free survival had indicated that some patients with advanced leukemia might be cured.^[87]

DISCUSSION

Bone marrow transplantation (BMT) offers curative potential for patients with high-risk hematologic malignancies, but the post-transplantation period is characterized by profound immunodeficiency. Recent studies indicate that the intestinal microbiota not only regulates mucosal immunity, but can also contribute to systemic immunity and hematopoiesis. Using antibiotic-mediated microbiota depletion in a syngeneic BMT mouse model, here we describe a role for the intestinal

flora in hematopoietic recovery after BMT. Depletion of the intestinal microbiota resulted in impaired recovery of lymphocyte and neutrophil counts, while recovery of the hematopoietic stem and progenitor compartments and the erythroid lineage were largely unaffected. Depletion of the intestinal microbiota also reduced dietary energy uptake and visceral fat stores. Caloric supplementation through sucrose in the drinking water improved post-BMT hematopoietic recovery in mice with a depleted intestinal flora. Taken together, we show that the intestinal microbiota contribute to post-BMT hematopoietic reconstitution in mice through improved dietary energy uptake.

Depletion of the Intestinal Microbiota Impairs Post-BMT Hematopoiesis:

To test the role of the intestinal microbiota in immune reconstitution after BMT, we performed syngeneic BMT (B6 / B6) after lethal irradiation in specific pathogen-free (SPF) mice with an intact intestinal flora and in mice treated with two different abx regimens: ampicillin + enrofloxacin (AE) and vancomycin + amikacin (VA) administered in drinking water. Ampicillin and enrofloxacin are both relatively well absorbed in the intestine (Eriksson and Bolme, 1981; Heinen, 2002), while vancomycin and amikacin both have poor oral bioavailability with negligible systemic effects (Jagannath *et al.*, 1999; Tedesco *et al.*, 1978). Both treatments reduced the colonic bacterial abundance 1,000-fold compared to untreated control mice. After BMT, we found a dramatic reduction in peripheral white blood cell (WBC) count recovery in mice treated by either of the abx regimens, while platelet (PLT) and red blood cell (RBC) counts were less affected. The reduction in total WBC count could be explained to some extent by lower counts of neutrophils and monocytes, but the most dramatic difference was a 3-fold decrease in lymphocytes. Abx-treated mice had lymphocyte counts below the normal range and 5- and 3-fold reductions in B and T cell lineages, respectively. Consistent with an impaired hematopoietic recovery, AE- and VA-treated mice also had lower bone marrow cellularity 28 days after BMT compared to untreated mice. Importantly, abx treatment also lowered WBC and lymphocyte counts in an allogeneic, minor-MHC-antigen disparate BMT model. To assess the functional implication of this lymphopenia, we infected mice intravenously with *Listeria monocytogenes* 21 days after BMT following a 3-day abx washout. AE-treated mice had higher bacterial load in the spleen 3 days after infection compared with untreated controls, indicating a functional immune deficit in the mice with a depleted intestinal flora.

Depletion of the Intestinal Microbiota Sensitizes Mice to Semi-Lethal Irradiation:

While survival after a lethal dose of radiation requires transplantation of unexposed donor bone marrow, hematopoietic reconstitution can also be modeled by sub-lethal irradiation and subsequent endogenous hematopoietic recovery without transplant. Depletion of the flora with the AE- or VA-abx regimen

sensitized mice to a 750 cGy semi-lethal irradiation dose 60% of untreated mice survived up to 60 days after irradiation, while all AE-treated mice and 90% of VA-treated mice died around day 24 after irradiation. All abx-treated mice had lower lymphocyte counts and VA-treated mice had lower neutrophil counts when compared to untreated mice 14 days after irradiation. The time of death (mean 24 days, range 18–31 days) indicated hematopoietic failure (Williams *et al.*, 2010) and moribund mice had an acellular bonemarrow (cells from both hindlegs totaled $3.3-7.3 \times 10^6$, which is less than 10% of the normal count) but no signs of infection or sepsis (no ascites or bacteria in peripheral blood or tissues). Furthermore, necropsy of AE-treated mice that were still alive 21 days after irradiation showed centrilobular hepatocellular atrophy and fatty change consistent with hypoxia due to prolonged severe anemia. Mice with a depleted flora also showed reduced hematopoietic regeneration in bone marrow vertebrae compared to untreated mice, possibly explaining the reduced survival of mice with a depleted flora after semi-lethal irradiation. Thus, abx-mediated depletion of the intestinal flora impairs both hematopoietic reconstitution after syngeneic BMT and autologous recovery after semi-lethal irradiation.

Impaired Post-transplant Hematopoiesis in Abx-Treated Mice Is Mediated by Flora Depletion: We next assessed whether the detrimental effect of abx treatment on hematopoietic reconstitution is mediated by the intestinal microbiota. The effects observed after oral administration of either absorbed (AE) or non-absorbed (VA) drugs suggested a microbiota-mediated effect. To verify that the impaired hematopoiesis was due to depletion of the microbial flora rather than a direct systemic effect of the abx, we utilized a colony of mice that harbor a beta-lactam-resistant microbiota by virtue of having been maintained for years under continuous abx administration (Caballero *et al.*, 2017). One group of mice was given a fecal microbiota transfer (FMT) of the resistant flora (Res-FMT), while control mice were given an FMT with normal flora from SPF mice (Ctrl-FMT). The mice within each group were then subjected to either AE treatment or no abx and underwent BMT. As expected, Res-FMT mice had sustained abundance of fecal bacteria despite AE treatment and 16S rRNA sequencing showed a diverse flora similar to that of the Res-FMT donor mice. Plasma concentrations of ampicillin and enrofloxacin were not lower in Res-FMT recipients, demonstrating that the transferred resistant flora was not degrading the abx. Bone marrow cellularity, WBC counts, and frequencies of lymphocytes and myeloid cells were effectively rescued in the AE-treated Res-FMT mice compared to AE-treated Ctrl-FMT mice, demonstrating that the impaired post-BMT hematopoiesis was due to depletion of the intestinal flora and not direct effects of the abx on hematopoiesis.

Depletion of the Intestinal Flora Impairs Lymphoid and Myeloid Differentiation: To further determine the effects

of flora depletion on post-transplant hematopoiesis, we analysed the hematopoietic stem and progenitor cell compartment 28 days after BMT. Despite an almost 50% decrease in total bone marrow cells, the number of long- and short-term hematopoietic stem cells (LT-HSCs and ST-HSCs) and multi-potent, common lymphoid, and common myeloid progenitors (MPPs, CLPs, and CMPs) was not consistently reduced in the mice with a depleted flora (AE-treated, VA-treated, or Ctrl-FMT mice treated with AE) when compared to mice with an intact intestinal flora (all untreated groups and AE-treated Res-FMT mice). The reduced total bone marrow cellularity could be explained by reduced numbers of both myeloid and B lymphoid cells, while reductions in T cells were observed as lower number of thymocytes. Closer evaluation of differentiating B cells in the bone marrow and spleen revealed lower numbers of all cell subsets, except transitional B cells in the spleen, as well as lower total splenic cellularity in the mice with a depleted flora compared to mice with intact flora. Similarly, all T cell subsets in the thymus were reduced in abx-treated mice compared to untreated mice. Thus, the effects of flora depletion on post-transplant hematopoiesis present mainly as changes in expansion and differentiation of more mature cells. Morphologic assessment of bone marrow smears revealed that the ratio of myeloid to erythroid cells was decreased in AE- and VA-treated mice compared to untreated BMT recipients. Similarly, reductions were observed in the granulocyte- monocyte progenitor (GMP) compartment in AE- and VA-treated mice compared to untreated mice while numbers of megakaryocyte-erythroid progenitors (MEPs), and as previously mentioned, RBC counts were not significantly perturbed in the mice with a depleted flora. In addition, *in vitro* colony-forming cultures showed equal frequencies of myeloid colony-forming units that, together with the reduced total bone marrow cellularity in mice with a depleted flora, indicated reduced numbers of GMPs. These results show that flora depletion impaired myeloid and lymphoid differentiation while largely sparing the stem and progenitor cell compartments and erythroid lineage. Thus, the flora is likely to influence expansion and differentiation steps in hematopoiesis. To assess an alternative hypothesis that initial homing of donor cells to the marrow was impaired by flora depletion, we analyzed bone marrow and spleen compositions 16 hr after injection of cells.

Sucrose Supplementation Improves Post-transplant Lymphopoiesis in Mice: with an Abx-Depleted Intestinal Flora Dietary restriction, both in experimental models and in human patients with eating disorders such as anorexia nervosa, has an impact on hematopoiesis, including reduced lymphocyte numbers (Elegido *et al.*, 2017; Tang *et al.*, 2016). To test if reduced uptake of calories in flora-depleted animals contributed to impaired post-BMT hematopoiesis, we supplemented the drinking water with 5% sucrose. Although not the form of energy usually provided to the host by the intestinal bacteria, sucrose is a simple carbohydrate absorbed directly by the

host without the aid of the flora. This concentration has been previously shown not to induce a preference for the water and to maintain a relatively constant consumption of chow (Lewis *et al.*, 2005), with an expected supplementation of approximately 0.9 kcal/mouse/day (about 7% of daily caloric intake). Sucrose supplementation increased peripheral WBC counts after BMT, and particularly increased the low lymphocyte count in AE-treated mice. Sucrose supplementation also increased bone marrow cellularity, bone marrow B cell, and myeloid frequency, although this did not reach statistical significance. In addition, sucrose-supplemented AE-treated mice had normalized thymocyte counts and showed a trend of increased periovarian VAT mass compared to non-supplemented AE-treated mice. Sucrose supplementation did not alter fecal bacterial abundance or significantly shift the composition of the remaining intestinal flora in AE-treated mice. Taken together, these data suggest that sucrose supplementation can compensate for decreased post-transplant lymphopoiesis due to loss of intestinal flora.

METHOD DETAILS Radiation and Bone Marrow Transplantation: Mice were given a split 1100cGy radiation dose and administered 5×10^6 bone marrow cells via tail vein injection. A single 750cGy radiation dose was used to test endogenous reconstitution. For analysis of homing, 5×10^6 CFSE-stained bone marrow cells or 2×10^4 LineageSca1+ ckit+ cells sorted from the bone marrow of GFP+ B6 mice (C57BL/6-Tg(UBC-GFP)30Scha/J) were injected via the tail vein and organs harvested 16h later. Complete blood counts from sub-mandibular bleeds were analysed by using a ProCyte Dx Hematology Analyzer (IDEXX Laboratories). Normal CBC ranges were based on data from the C57BL/6 North American Colonies (Charles River, 2012).

Drug Treatments and Sucrose Supplementation: Ampicillin 0.5 g/L and enrofloxacin 0.25 g/L or vancomycin 0.5 g/L and amikacin 0.5 g/L, were given in the drinking water starting 5 days before BMT and throughout the experiment. Metronidazole 0.5 g/L, streptomycin 5 g/L, or aztreonam 0.5 g/L were started 9 days before BMT and administered throughout the experiment. Ampicillin 0.5 g/L and enrofloxacin 0.25 g/L treatment without BMT was administered in the drinking water for 35 days. Abx water was changed at least every 5-7 days. Sucrose was supplemented (50 g/L) in drinking water and changed every 2 – 3 days.

SUMMARY

Fifty years have gone by since the first experiments in mice that were to lead to the wide application of human hematopoietic cell transplantation. The solution of problems recognized in human patients came from animal research ranging from mice to dogs to non-human primates. In the early days these cellular transplantations were carried out only in terminally ill patients. Now in many diseases transplantation is carried out early in the

course of the disease with greatly improved results. Research designed to continue improvement of the application to human patients includes the achievement of engraftment without lethal marrow ablative regimens, the use in autoimmune diseases, *ex vivo* culture of hematopoietic stem cells, gene transfer studies, and the development of techniques for inducing tolerance for solid organ grafting. The development of effective antiviral and anti-fungal drugs and the shift to outpatient care has resulted in dramatic reduction of the cost of transplantation as well as improved long-term survival. Pending the development of highly specific curative agents, hematopoietic cell transplantation will continue to be used increasingly during the early years of the third millennium.

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