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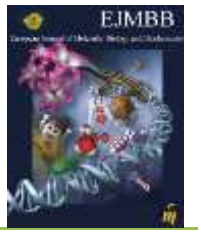

EXPLORING THE ROLE OF PRIMITIVE STEM CELLS IN RECEPTOR EXPRESSION MANIPULATION FOR CYTOTHERAPY IN APLASTIC ANEMIA: INSIGHTS FROM CHEMOTHERAPEUTIC DRUG EFFECTS ON SCA-1+ HEMATOPOIETIC STEM CELLS

Sreshmitha Manchala*

Assistant Professor, Department of Biochemistry, Sree Balaji Medical College & Hospital, Chennai – 600044, Tamil Nadu, India.

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ABSTRACT

All blood cell lineages are derived from hematopoietic stem cells (HSCs) through a process known as hemopoiesis, wherein these stem cells exhibit self-renewal capabilities. As murine HSCs differentiate, their expression of Sca-1, along with other mature cell markers, diminishes, indicating their transition towards a more specialized state. Throughout an individual's lifespan, Sca-1+ HSCs within the bone marrow sustain the pool of stem cells necessary for hematopoiesis. Aplastic anemia, characterized by a decline in the production of healthy blood cells, is believed to stem from a primary deficiency in stem cells. Our laboratory studies have shown that during experimental drug therapy, specifically with chemotherapeutic agents like Busulfan and Cyclophosphamide, Sca-1+ BM-HSCs are significantly impacted. Elevated levels of Caspase-3 activity and Annexin-V positivity in the bone marrow indicate a heightened susceptibility of hematopoietic stem cells to premature apoptosis. This susceptibility is more pronounced in Sca-1bright BM-HSCs compared to Sca-1dim BM-HSCs. Scanning electron microscopy (SEM) reveals a disrupted microenvironment within the marrow, which correlates directly with the decline in cell population and receptor expression. Based on the aforementioned experimental findings, it is proposed that primitive stem cells may have a role in the regeneration of the bone marrow. Manipulating receptor expression for cytotherapy in aplastic anemia patients could potentially leverage these primitive stem cells for therapeutic intervention.

INTRODUCTION

All blood cell lineages are replenished throughout life by multipotent self-renewing HSCs [1, 2]. A sophisticated method has been developed to isolate HSCs, which can be studied in vitro despite making up only 1 out of 10,000 bone marrow cells. Mouse multipotent clonogenic HSC can be isolated from a Population of primitive HSCs using nearly pure genes Sca-1 as a marker [3, 4], which is HSCs from adult murine mice are commonly identified by this method [5, 6]. The hematopoietic microenvironment regulates In a complex, Sca-1 is expressed manner.

Corresponding Author
Dr. Sreshmitha Manchala,

A decrease in Sca-1 expression occurs during the differentiation of HSCs into lymphoid or myeloid progenitors. Historically, Microenvironment inducing hematopoiesis [7-12] or "Stem Cell niche" [13] has been considered necessary to maintain a population of primitive Bone marrow contains Sca-1+ cells. Additionally, the stem cell niche maintains hematopoiesis by tightly balancing self-renewal and differentiation, while also maintaining a quiescent stem cell pool [14, 15]. In order to maintain proliferation, differentiation, and survival, hematopoietic growth factors are secreted in large quantities.

It is hypoplastic or hyperplastic marrow failure that results from the dysfunctional hematopoietic inductive microenvironment, of which malignancies

impair the equilibrium How HSCs differentiate and self-renew [16]. There are many diseases that have an empty bone marrow and the inability to Anemia or hypoplastic bone marrow failure, which cannot produce healthy mature blood cells [17-19]. There are many theories about the causes of this disease, ranging from a deficiency in primitive stem cells to a disorder of the stem cell niche. As a result of immunologically-mediated cell death, bone marrow failure is most often associated with Aplastic anemia [20, 21]. The hematopoietic compartment of the bone marrow is also strongly suggested to be prone to premature apoptosis. The evidence of primitive stem cells and the HSC niche being involved in Aplastic anemia is still ambiguous, despite many reports regarding stem cell dysregulation.

Through flowcytometry This study evaluates the apoptosis and fate of Sca-1+ primitive HSC populations and their microenvironment in bone marrow using free radical counting Analyzing FCM and caspase-3, scanning electron microscope, and light microscopy microscopy (SEM). During cyclophosphamide and busulfan treatments, experimentally induced Aplastic anemic mice are subjected to phenotypic analysis/SEM evaluation.

MATERIALS AND METHODS

The whole hemogram profile was determined by the number of neutrophils, WBCs, reticulocytes, platelets, and hemoglobin percent. In order to detect apoptosis, BM-HSCs (1X106) During incubation at 37°C in the dark, the cells were cultured for 30 minutes million units of (BD-Bioscience, USA) A detectable anti-mouse Sca-1 monoclonal antibody was PE-labeled along with 5Isotype Controls. million units of Labeled with FITC, this antibody is anti-mouse Annexin V (dilutely in binding buffer). PBS was then used to remove excess fluorescence. Analysis was conducted with CellQuestpro software using the BD-FACS Callibur. Caspases-3 intracellular activity of BM-HSCs was assessed using a caspase-3 colorimetric assay kit. Using a water bath, 50L of It was applied by mixing lysate with 2x buffer/DTT mix and 1 mM Caspase-3 substrate (DEVD-pNA), discarding the pellet, and collecting the supernatant. incubated for an hour at 37C. Conjugated substrate was not present in the negative control reaction. A microplate reader was used to read the samples at 405 nm.

RESULTS

Blood clots in the peripheral circulation. Standard laboratory techniques were used to measure hemoglobin, reticulocytes, white blood cells, polymorpho nuclear neutrophils, and platelets to determine the clinical status of the disease. Aplastic groups (0.18%) showed significantly lower reticulocyte counts than normal groups (0.73%–1%) while hemoglobin levels were low (Table 1). There were significant differences between the diseased and normal groups in the total WBC and neutrophil counts (WBC 2.7 × 103/μL, Neutrophil 7.35%, respectively) in comparison with the norm (WBC 6.2 × 103/μL, Neutrophil 22.75%). Aplastic anemia-induced mice show significantly downregulated Sca1+ populations based on phenotypic characterization of BM-HSCs expressing Sca1. The gated cells in R2 were only 0.01% of those in normal, as opposed to 12.23% in normal. Likewise, the Sca1dim population is represented by the R1 region; in Aplastic anemia, 2.30% of Sca1dim cells are present compared to 8.83% in normal.

(b) BM-HSCs exhibiting Annexin-V positivity (3.05%) in Aplastic anemia were significantly more positive than those in normal blood (0.46%). BM-HSCs expressing Sca-1+ Annexin-V indicate premature senescence within the primitive bone marrow of an Aplastic anemia patient. As compared to the normal control (2.6%) of BM-HSCs, BM-HSCs were significantly more active at caspase-3 (49%) in Aplastic anemia, further confirming the premature apoptosis of bone marrow HSCs. Flowcytometric analysis has already confirmed the Primitive Sca1+ populations have decreased significantly as a result.

A bone marrow smear study showed the same bone marrow architecture in normal and experimental groups. In comparison with the normal marrow, where the cells are densely packed, Aplastic anemia has a large number of fat cells (adipocytes) in the marrow.

TABLE 1: Depressed haemoglobin levels in peripheral blood.

Aspects	(Mean + SD) Normal control groups	The mean and standard deviation for aplastic anemia groups
(g/dL) Hemoglobin	16.98 ± 0.25	16.98 ± 0.25
Whte Blood Cells (x 103/μL)	7.1 ± 2.27	3.8 ± 0.67
Red Blood Cells (x 106/μL)	9.43 ± 0.31	4.7 ± 0.87
Blood platelets (per 106 liters)	433 ± 14.81	199 ± 15.35
% of reticulocytes	0.88 ± 0.14	0.17 ± 0.06
Amount of neutrophils (%)	23.74 ± 3.20	8.34 ± 3.74

DISCUSSION

A range of aryl hydrocarbons and alkylating agents have been shown to cause bone marrow failure by directly damaging stem cells and decreasing the production of mature blood cells. The peripheral blood hemogram demonstrated damage rather than failure of the bone marrow on the basis of the combination of busulfan and cyclophosphamide that we used in our experiment [22]. It is believed that busulfan and cyclophosphamide induced chronic hypoplastic marrow failure through pancytopenia, thrombocytopenia, hemoglobin deficiency, and a lesser number of reticulocytes. The peripheral blood started showing hypoplastic marrow failure effects around 9 weeks after the injection of chemotherapy drugs, which was evident at around 12 weeks (time of our study). As a result of bone marrow failure, the peripheral blood profile is scanty, indicating that a damaged stem cell population remains. When a stem cell proliferators is impaired, moderate marrow failure results in death, and severe marrow failure results in organ failure.

A Sca-1 receptor expression pattern was investigated to define "residual injury" in BM-HSCs. It was observed that bright and dim Sca-1+ primitive BM-HSCs expressed typical receptor patterns in the presence of Aplastic anemia. Aplasia Animals show more Sca-1bright positivity (0.01%) than normal animals (12.23%) and less Sca-1dim positivity (2.30%) than normal animals (8.83%). In the bone marrow, cyclophosphamide and busulfan induce damage to the primitive cell population [23], which explains the differential receptor expression. The Sca-1bright stem cell population is the “more primitive” and the Sca-1dim denotes the “less primitive” population. Though both populations are in quiescent states from chemotherapeutic damage, they are able to resist themselves a little more due to their maturity level. The combined chemotherapeutic drug induced bone marrow cellular damage is significantly correlated with Sca-1dim and Sca-1bright receptor expression and maturity level. In contrast to normal healthy bone marrow, a bone marrow smear study revealed that Aplastic anemia patients have large fat cells (adipocytes) surrounded by empty spaces. An electron microscope image of aplastic bones revealed a lack of cells in the bone marrow, which resembled hollow cavities [24]. As a result of both chemotherapeutic induction and increased caspase-3 activity, the hollow cavities reflected the premature senescence pattern. By deranged marrow structure, and "pinhole" views of the marrow microenvironment, busulfan and cyclophosphamide cause massive destruction of stem cells in the BM, leading to hypoplasia, peripheral pancytopenia, and adverse effects on the bone marrow microenvironment. Additionally, the SEM results and increased caspase-3 levels showed that Sca-1bright BM-HSCs were severely affected more than Sca-1dim, that is, less primitive BM-HSCs.

CONCLUSION

HSCs play a crucial role in maintaining blood cell lineages and their susceptibility to chemotherapy. Experimental drug treatment has a significant impact on Sca-1+ BM-HSCs, leading to apoptosis and elevated Caspase-3 activity. An important difference between Sca-1bright and Sca-1dim BM-HSCs is their susceptibility to apoptosis. Aplastic anemia patients have also been shown to have altered marrow microenvironments associated with decreased cell populations. Overall, these insights emphasize the complexity of HSC regulation and their importance in disease states like aplastic anemia. In the future, bone marrow regeneration strategies and manipulation of receptor expression may provide promising avenues for improving therapeutic outcomes.

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