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Polyclonal CD5+/CD19+ B1a lymphocytes after allogeneic stem cell transplantation: a potential diagnostic pitfall

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ABSTRACT

In adults, B-lymphocytes comprise approximately 10% of circulating lymphocytes. The majority of peripheral B cells are B2 cells ("Mature" B-cells), which function as part of the humoral adaptive immune system. B1 cells ("Innate-like" B cells) are another sub-class of B lymphocytes, considered as innate immune cells with a characteristic phenotype (CD20+, CD27+, CD43+, CD70-, CD11b+, sIgM++, sIgD+) which can be divided into two subtypes; B1a (CD5+): spontaneously produce broadly reactive natural IgM, and B1b (CD5-): can generate T-cell independent, long-lasting IgM. There is very limited data available, indicating a correlation between allogeneic bone marrow transplantation and an increase in B1a cells. Here we present a case of a 17-year-old female with homozygous sickle cell disease (HbSS disease) who underwent hematopoietic stem cell transplant (HSCT). Approximately seven months post-transplant, she was found to have 16% immature mononuclear cells on complete blood count (CBC)-differential report. A follow-up peripheral blood flow cytometry showed that these cells were polyclonal CD5+/CD20+ B-cells, and comprised 66% of lymphocytes. Further workup and follow up failed to reveal any lymphoproliferative disorders. It is important not to misdiagnose these cells as an atypical CD5+ lymphoproliferative disorder. The presence of B1a cells has not been widely reported in non-neoplastic post-stem cell transplanted patients. This case also adds to and expands our knowledge regarding the presence of increased circulating B1a cells after stem cell transplant in a patient with no history of hematological malignancy.

Keywords

Flow Cytometry, Hematology, Pathology, Stem Cell Transplantation

INTRODUCTION

B-lymphocytes constitute approximately 10% of peripheral blood lymphocytes. The majority of peripheral B cells are B2 cells, which function as part of the humoral adaptive immune system by secreting antibodies. A very small subset of B cells is described as B1 cells, which are distinguished from B2 cells by their unique tissue distribution, distinct cell surface phenotype, capacity for self-renewal, and prominent role in natural antibody production.

• **Mature B cells ("B2 cells"):** The majority of mature B cells that are found in adult lymphoid organs and that arise later in ontogeny, are called B2 cells or conventional B cells.¹ In adult humans, the bone marrow produces B2 cells throughout life, which are negatively selected by recognition of self-antigens and further develop in secondary lymphoid tissues. B2 cells function as part of the adaptive humoral immune system as well as antigen-presenting cells (APCs). They are present in the blood and the secondary lymphoid tissues. These cells give rise to either follicular B cells or marginal

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zone B cells in the spleen, which can respond to a broad range of T-dependent and T-independent antigens. B2 cells provide antibody-mediated protection against various pathogens. They undergo class-switching and somatic hypermutation that leads to the generation of affinity-matured memory cells;²

• **Innate-like B cells (“B1 cells”):** The fetal B lymphocytes were first described by Hayakawa K et al. in 1985 in mice.³ They showed that a distinct subset of B-cells has the capacity to regenerate immunoglobulin M-expressing “Ly-1” B cells, now referred to as B1 cells, which function as intermediaries between innate and adaptive immune system.⁴ B1 cells constitute a distinct B cell lineage with a unique set of characteristics and represent a small subset residing within a dominant B cell population. There is an extremely wide variation in the frequencies of B1 cells in the peripheral circulation of normal individuals (the range of “normal” B1 cells frequencies may span less than 1% to greater than 9% of circulating cells).⁵ These cells are classified as a part of the innate immune response because they have no memory. During fetal and neonatal development, B1 cells make up the predominant population of lymphocytes (60-80% of B cells in the umbilical cord blood)⁶, but decrease in the peripheral blood with age.⁷ In adults, they migrate mostly to peritoneal and pleural cavities upon generation from the stem cells and maintain their existence by self-renewal of pre-existing cells. The process of self-renewal declines with age.⁸ Upon activation, B1 cells can recirculate through secondary lymphoid tissues and secrete antibodies.⁹ B1 cells produce “natural” immunoglobulin M (IgM), which is largely encoded by germline immunoglobulin genes. The natural IgM immunoglobulins produced by B1 cells are poly-reactive, and act in the neutralization of pathogens, before the body develops the high-affinity antibodies. These immunoglobulins are the first line of defense against pathogens such as polysaccharide-encapsulated bacteria¹⁰ and also cross-react with epitopes expressed on dead and dying cells.¹¹ A second, parallel function of B1 cell natural antibody lies in housekeeping or homeostatic activity that speeds elimination of dead and dying cells and cellular debris.¹² B1 cells also can serve as a barrier immunity by class switching to IgA to control microbes at mucosal surfaces.¹³ B1 cells have characteristic phenotype of CD20+, CD27+, CD43+, CD70-¹³, CD11b+, sIgM++, sIgD+ and can be divided into two subtypes:¹²

- **B1a (CD5+):** Spontaneously producing broadly reactive natural IgM and IgA.
- **B1b (CD5-):** These B1b cells bear all B1a cell surface markers except CD5, are regulated separately from B1a cells, and appear to develop in concert with B2 cells.¹² B1b cells can generate T-independent, long-lasting IgM responses to some infectious pathogens.¹⁴

B1a Cells

CD5+ B1 cells (B1a cells) are primarily found in the peritoneal cavity and develop as a self-replenishing population. Some studies showed that B1a cells in an

adult could compromise 5-30% of B cells in the blood,¹⁵ 10-30% in tonsils,¹⁶ and approximately 1-10% of B cells in the spleen.^{4,15} There is increasing evidence correlating the weak auto-reactivity of the B1a cells and its role in the autoimmune pathogenesis such as rheumatoid arthritis, Sjogren’s syndrome, and systemic lupus erythematosus.¹⁷ The increase of B1a cells also has been reported in some patients with HCV hepatitis.¹⁸ Some authors have described these B1a cells as CD5+ normal stage 3 hematogones, which can increase after a bone marrow transplant.¹⁹ There are very few data available indicating an increase in the B1a cells population following allogeneic bone marrow transplantation in adults with hematological malignancies.²⁰ Some studies have demonstrated that the number of polyclonal CD5+ B cells (B1a cells) can increase up to 20% of total cells, even after 18 months of transplantation,²¹ and subsequently return to the normal level after two years’ post-transplant.²² It is important not to confuse it with an atypical CD5+ lymphoproliferative disorder²³ or other condition with circulating clones of B cell with surface markers similar to chronic lymphocytic leukemia (CLL).²⁴ To our knowledge, these changes have not been reported in the literature in patients with non-hematological malignancies who underwent a bone marrow transplant. The following case shows that the increase of these CD5+/CD20+ cells (B1a cells) can occur in patients without any history of hematological malignancy. This further supports the reactive nature of this phenomenon as opposed to a neoplastic or preneoplastic process.

CASE REPORT

A 17-year-old female with a history of Sickle cell disease (Hb SS disease) received a hematopoietic stem cell transplant (HSCT) from an unrelated donor due to her transfusion-related iron overload, and abnormal transcranial Doppler (TCD) findings. The post-transplant course was complicated with graft versus host disease (GVHD) in skin and lungs, human herpesvirus 6 (HHV6) reactivation, and oral/labial herpes simplex (HSV) infection.

Approximately seven months after the HSCT, she was found to have 16% of immature mononuclear cells on a complete blood count (CBC) differential report. A follow-up peripheral blood flow cytometry study showed a B cell population with a dim expression

of CD5, which comprised 66% of lymphocytes (Figure 1A and 1B). Other T cell markers (CD3, CD4, CD8, CD2, and CD7) were negative (Figure 1C, 1D, and 1E). Negative CD23 expression with positive FMC helped

to rule out chronic lymphocytic leukemia (CLL) (Figure 1F). No evidence of a monoclonal B-cell population was identified by kappa and lambda analysis (kappa/lambda ratio of 2.3:1 (Figure 1G)).

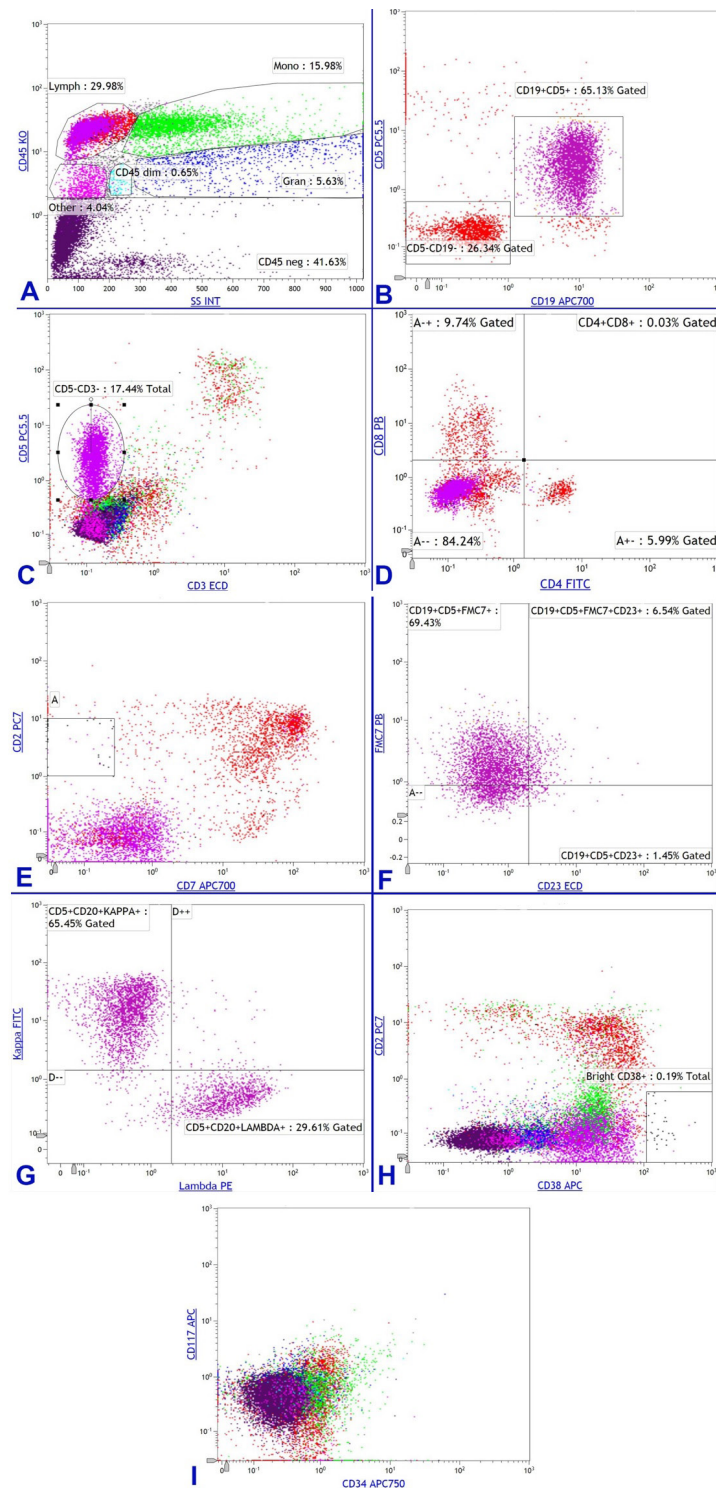


Figure 1. Peripheral blood flow cytometry shows that ~30% of cells are comprised of lymphocytes (A), and ~66% of lymphocytes show dim expression of CD5; CD19+CD5+ population highlighted in pink (B). These CD19+CD5+ cells did not show any T-Cell markers; CD3- (C), CD4-CD8- (D), CD2-CD7- (E) These cells show positive FMC without expression of CD23 (F) which argued against the possibility of chronic lymphocytic leukemia (CLL). No monoclonal expression of kappa or lambda was identified; kappa/lambda ratio of 2.3:1 (G). These cells also did not show plasmacytic bright CD38 expression (H) or immature lymphocytic markers; CD34-CD117- (I).

These cells also lacked plasma cell markers (bright CD38, CD138 (Figure 1H)), and immature markers (CD34 and CD117) were also negative (Figure 1I). These cells also did not express CD10, CD15, and CD56. These findings were interpreted as a reactive process; therefore, no further treatment or workup was performed. A repeat peripheral blood flow cytometry performed 22-months post-transplant demonstrated a persistent population of B1a cells, comprising 18% of lymphocytes. Chimerism studies performed at that time were noted to be 99-100% donor.

DISCUSSION

B1 cells are a distinct type of B cells, derived from hematopoietic stem cell (HSC) that are rare or missing in adults,²⁵ which act as tissue-residing innate-like B cells. Unlike mature B cells (B2 cells), which maintain by continual replacement with newly generated cells from the bone marrow, B1a cells maintain their existence by renewal process after birth. They usually are present in the peritoneum and act as part of the innate immune system by making natural poly-reactive IgM and IgA. The increase of these CD5+/CD20+ cells has been reported in a few patients who underwent bone marrow transplant for hematological malignancies.^{18,19} Distinguishing B1a cells from neoplastic B-cells can be problematic in these patients. However, this phenomenon has not been observed in patients who have received bone marrow transplant due to a non-hematological malignancy. Our case demonstrates that B1a cells can be found in increased numbers in the peripheral blood (66% of lymphocytes in our study) and also persisting for almost two years after transplant. The reason for the increase and persistence is not well understood, but may be related to autoimmunity and/or a chronic graft vs. host disease state. However, as stem cell transplant is used as a curative therapy in patients with hematologic diseases, it is important to distinguish these physiological B1a cells from a recurrent or secondary hematologic malignancy. By flow cytometry, this can be accomplished by carefully evaluating the CD19+/CD5+ B-cells by their fluorescence patterns; Normal physiologic B1a cells show a polyclonal CD19+bright/CD5+dim-to-bright pattern. For example, chronic lymphocytic leukemia (CLL) typically shows monoclonal CD19+dim/CD5+dim expression. By recognizing the existence of these

reactive B1a cells, unnecessary diagnostic tests (e.g., bone marrow aspiration) or aggressive therapies can be prevented in post-transplant patients.

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