Writer: Maya Chaddah
Editor: Lisa Willemse
Reviewer: Armand Keating

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Blood Disorders

Research is a dynamic activity that creates new ideas. It provides a forum for generating observations and testing why they occur. Because people and their diseases are so diverse, clinical trials are the ONLY WAY it is possible to test whether new ideas about how to diagnose or treat human disease will work. But the process of taking research from bench to bedside is a lengthy one and demands not only vision but also years of teamwork and dedication on the part of scientists, physicians and patients. This document presents basic information about blood disorders and frames the context for the discussion that follows about how lessons learned from stem cell research may help fight thesediseases. Readers may also wish to peruse additional web resources or speak with their physicians for more information about this condition.

About Blood Disorders

The blood is composed of many different cells and disease can strike any one of them. Before delving into the application of stem cells as potential therapies, we first present the basics of blood development. The cells circulating in our blood are made by hematopoietic stem cells (HSCs) that live in the bone marrow. Every day HSCs churn out a staggering number of cells – upwards of seven billion – to replenish the red and white blood cell-sthat are exhausted through normal wear and tear. This process is called hematopoiesis. Some of the cells produced by HSCs stay in the bone marrow but many enter the blood where they remain for life. Others leave the bone marrow and travel via the blood to make the tissues their home. After decades of studying the process of hematopoiesis, scientists can now track the fate of HSCs as they mature and diversify to form the cells that work in the bone marrow and blood every day of our lives.

As we learn more and more about blood development, the model that describes how stem cells mature is refined. However the very initial steps are quite predictable: stem cells either renew themselves or mature into more specialized cells,called progenitors. These progenitors, being one step further down the line of development, are more restricted in their ability to make other cells. In the case of HSCs, once they start to mature, their progeny no longer make HSCs but instead they make other more specialized blood system cells. The earliest progenitors of an HSC are categorized as being either ‘myeloid’ or ‘lymphoid’, and the cells that they make are said to belong to either the myeloid or lymphoid lineage. A lineage is simply a group of cells that share a common ancestry.

The cells in the myeloid lineage that we are most familiar with are erythrocytes (red blood cells) and megakaryocytes (large cells that make platelets). Erythrocytes carry oxygen to our tissues and shuttle carbon dioxide to the lungs where it is released when we breathe. Platelets are formed when megakaryocytes shatter into tiny particles, and as the platelets stick together they form blood clots. There are a number of other cells belonging to the myeloid lineage, such as macrophages, dendritic cells and granulocytes and, although less well known, these cells are just as important. Among other roles, they are involved in the very early, frontline immune response against general pathogenicthreatsand also help to kick start the ‘big guns’ of the immune response that form the other blood lineage, called the lymphoid lineage. These cells are commonly referred to by initials -- B, T, NK and NKT cells and they work by targeting assaults on the body that come from specific pathogens or cancers.

There are too many blood diseases to review the basic biology and the application of stem cells for each in detail here. Instead, we use broad strokes to present the general causes of blood disorders and then highlight certain

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types of stem cells, to be used either as current or future treatments or for better understanding the biology of blood diseases.

Causes

Blood diseases, also commonly called hematological diseases, can arise in any of a number of different cells belonging to the myeloid or lymphoid lineages. The causes are typically associated with congenital (inborn) deficiencies, immune deficiencies, autoimmune mechanisms, and cancer.

Genetic conditions that exist at birth are known as congenital, and may be the result of specific mutations within a gene, the absence of part or all of a gene (deletion) or the presence of too many of a particular gene (duplication). For example, sickle cell anemia is caused by a single mutation in the b-hemoglobin gene. The result is a twisted hemoglobin protein that impedes red blood cells from efficiently carrying oxygen to the tissues. Thalassemia is another inherited blood disorder affecting the production of red blood cells. In this disease, mutations or deletions in the genes that make hemoglobin are the culprits and the symptoms are very broad, ranging from stillbirth to children with jaundice, anemia, fatigue and even bone deformities.

Immune deficiencies of the blood, whether they are inborn or result later in life from infections, drugs or surgeries, affect the immune system and compromise our ability to stave off infections. There are many different types of immune deficiencies and they range from being mild to fatal in severity. You may remember David Vetter, the 'boy in the bubble' who was born in 1971 with a severe combined immune deficiency (SCID) that left him without the normal numbers of T and B cells. This meant that he was unable to fight off infections – even the ones that occur in our body all the time that never make us feel sick. Only living in a sterile bubble could protect David from these life-threatening infections but sadly he died at the age of 12 after emerging from the bubble for an emergency medical treatment.

Autoimmune diseases also involve the immune system, but in this case the problem is that the immune cells themselves have a misguided sense of what to attack. Instead of fighting foreign pathogens that invade our bodies, the cells of the immune system are somehow triggered to attack the body's own tissues, wreaking havoc that can last a lifetime. Autoimmune diseases of the blood are few. One example is a platelet-attacking condition called immune thrombocytopenia purpura. Because people with this disease have very few platelets, they are unable to clot blood normally and the skin may become tinged purple as a result of blood leaking out of the vessels.

Last on the list of causes of blood disorders is perhaps the most talked about and researched in the modern world: cancer. Cancers of the immune system can arise in any number of cells in the blood system, from the hematopoietic stem cell all the way to the mature cells of the myeloid or lymphoid lineages. Leukemias are cancers that exist in hematopoietic cells residing in the bone marrow and blood. Depending on the cell type affected, they are labeled as either myeloid leukemias or lymphoid leukemias. Myelomas are cancers arising in plasma cells (specialized B cells that produce antibodies which stick to pathogens and mark them for destruction). Myelomas not only overwhelm the bone marrow compartment but also spew out abnormal antibodies that can lodge in the kidneys. Patients with this cancer may go on to experience anemia, kidney failure, high calcium levels and bone lesions. Solid cancers that form in cells of the lymphoid lineage – most commonly T or B cells– are called lymphomas. Although typically found in the lymph nodes, lymphomas may also grow in other sites such as the spleen or thymus. As they grown and/or spread, lymphomas impinge on adjoining tissues and organs in the vicinity and affect their ability to function properly.

Treatment

The most common therapies for treating blood disorders include blood transfusions, drug therapies, and hematopoietic stem cell transplants (HSCT; commonly known as a bone marrow transplant). Transfusions are an immediate source of blood components and cells that may be lacking in a patient. Drugs such as erythropoietin (EPO) help to stimulate the patient's natural red blood cell production while others, such as rituximab, destroy cancerous B cells in leukemia and lymphoma.Hematopoietic stem cell transplants (HSCTs) are intended to have a more long-lasting effect because they supply a ready source of stem cells that can drive the production of new, healthy red and white blood cells in the body.

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How Can Stem Cells Help?

HSCs are uniquely positioned as a cell therapy for blood disorders because their normal job in the body is to make all the cells that populate the bone marrow and blood. Of all the stem cells currently studied, hematopoietic stem cells have the longest history of clinical use. The first hematopoietic stem cell transplant (HSCT) was performed in the 1950s in an attempt to cure a lethal form of leukemia. The premise was to wipe out the leukemic cells and normal bone marrow with radiation and then to transplant normal hematopoietic stem cells to rebuild the blood system. In the 20 years that followed the early trials, scientists learned the importance of finding the best donor-recipient match, and how to treat graft versus host disease and other complications arising from the transplant procedure.

Today, scientists are building on the wealth of knowledge gained from the long treatment history of HSCT. With the identification of many new sources of stem cells,they are exploring how they might be used to provide relief for people with blood and other diseases. Perhaps the most sought after application for stem cells is creating patient-matched cells as transplant material for cell replacement therapies for conditions where a class of cells or component of a tissue is lost, such as in Parkinson's disease, Type 1 diabetes, corneal injuries, liver disease, or muscular dystrophy – to name but a few. While this goal is certainly being pursued, many factors must still be optimized before some types of stem cells can be safely used in humans. In the meantime, scientists are putting stem cell technologies to good use by developing patient-derived stem cell lines that allow them to dig deeper into the biology of blood diseases and to screen drugs for their benefits or toxicity.

Canadian contributions

Canadians scientists have played an illustrious role in the development of stem cell research. Drs. James Till and Ernest McCulloch were the first in the world to prove the existence of stem cells. Dr. McCulloch was interested in bone marrow transplantation for cancer patients and together with the expertise of physicist Dr. Till they performed a pivotal series of experiments in the 1960s using radiation and bone marrow transplantation in mice. Not only did they prove that stem cells exist, but in teaming up with two other Canadian scientists, Drs. Louis Siminovitch and Andrew Becker, they also described the key function of stem cells being 'self-renewal' to form more stem cells and 'differentiation' to make specialized cells. These experiments set the stage for bone marrow transplantation for treating bone marrow failure in patients with blood cancers and aplastic anemia (a condition where both red and white blood cell lineages are depleted). In the late 1970s, Till and McCulloch went on to study leukemia and their experiments essentially paved the way for the idea that a small number of cancer stem cells present in a tumour contribute to its formation.

Another 'first' in the history of stem cell research came in 1994 when Canadian scientists led by Dr. John Dick at the University Health Network discovered cancer stem cells in leukemia. By 1997, the group was able to show that tumour activity from tissue containing cancer stem cells was transplantable and could initiate leukemia in mice. A very important human-mouse model was developed by this same group: they inserted a cancer gene into a human stem cell to seed the human form of leukemia into laboratory mice. This model allowed them to track the development of human leukemia, from the original cancer stem cell into cells of the blood lineage.

With this model in hand, the field has moved forward with a better understanding of normal and leukemic stem cells and the insights are being applied to the progression of symptoms in leukemia patients. Dr. David Kaplan at the Hospital for Sick Children is among one of the many Canadian scientists who are building on the cancer stem cell theory to identify potential anti-cancer stem cell drugs to treat leukemia and other blood cancers. Cancer stem cells are thought to be partially responsible for the recurrence of cancer following chemotherapeutic and radiation therapies, so killing these cells might lead to longer remissions, if not cures.

Research directions and clinical directions

Hematopoietic stem cells (HSCs)

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In the past 40 years, hematopoietic stem cell transplantation (HSCT), also commonly referred to as bone marrow transplantation, has become the most successful of all cell therapies. In terms of treating blood disorders, HSCT has been used with some success for thalassemia, immune deficiencies, leukemias and lymphomas. HSCT is also routinely used to restore the blood system of patients undergoing aggressive doses of chemotherapy for blood and other cancers.

HSCT can be autologous (from the patient) or allogeneic (from a donor). Autologous HSCT circumvents the problem of graft rejection but not all patients are candidates for this approach for a variety of reasons that may include age, weak health, or bone marrow disorders. The biggest stumbling block to the more widespread use of allogeneic HSCT is the availability of suitable donors. The need to 'match' a donor graft as precisely as possible to the recipient's constitution is the main issue and because matches are mostly imprecise the prolonged use of immunosuppressive drugs may become necessary to control graft versus host disease (GVHD). GVHD occurs because the transplanted donor immune cells recognize the patient's [recipient's] cells as foreign and mount a vigorous response to attack them. This can happen very quickly (acute) or over a longer period of time (chronic). Manifestations include skin rashes, diarrhea, inflammation of the membranes lining the digestive tract, destruction of the liver and lungs and account for the majority of deaths following allogeneic stem cell transplantation. Despite the possibility of causing GVHD, the donor immune cells also have the ability to kill tumour cells in the recipient. This is referred to as a graft versus leukemia effect (GVL) and is an important way that allogeneic HCT can cure the leukemia.

Although considered to be an aggressive form of therapy, today, HSCT can be used to treat a variety of different blood disorders, but despite the clear successes, upwards of 50% of patients are still not cured of their disease. In addition to the problem of graft versus host disease and infectious complications, some researchers theorize that a small population of stem cells called 'cancer stem cells' may be the reason that initial treatment successes so often end in relapse.

Cancer stem cells

The concept that cancers are fuelled by a small subset of cells with stem cell properties has existed for more than a century. This concept is extremely important because it implies that these cells must be eliminated to achieve cures. Butthe rarity of stem cells makes it difficult to identify and study them. The recent development of methods to isolate and characterize cancer stem cells has provided a new impetus to the field of cancer research, and a critical contribution has also been the creation of mice that lack an immune system and can serve as long-term hosts for primary sources of human cancer cells. Another advance has been the development of methods for separating different subsets of cells without killing them, so they can then be tested for their ability to generate a tumour in a transplanted mouse lacking an immune system.

Cells containing tumour-initiating activity, dubbed 'cancer stem cells' have been found in a number of different human cancers. Acute myeloid leukemia was the first disease in which researchers demonstrated that malignant cells transplanted into immune deficient mice would grow new tumours. Since then, cancer stem cells have also been identified in breast, brain, prostate, and colon cancer. The research field is convinced that chronic myeloid leukemia is caused by cancer in a very early hematopoietic cell or stem cell. Interestingly, there is also growing evidence that the cells that can generate new tumours are not necessarily normal stem cells that have become cancerous, but may represent "later" cell types that have reactivated stem cell properties.

As a pre-requisite to developing therapies targetingblood cancer stem cells, many questions must first be answered. How do cancer cells acquire "stem cell" properties? How are they different from the normal cells of the same tissue? Which signaling pathways can keep cancer stem cells quiet (non-dividing) but still alive, and which ones unleash uncontrolled growth? These represent but a smattering of the many questions being explored. The results should help pinpoint a way of targeting blood cancer stem cells while sparing normal stem cells in the body.

Peripheral blood stem cells

Low levels of hematopoietic stem cells can be found in the blood of healthy individuals. These are called peripheral

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blood stem cells. In the 1970s, scientists discovered that peripheral blood stem cells are also present in the blood of patients with myeloid leukemia. Through the 80s, they learned that they could harvest peripheral blood stem cells in patients with leukemia, lymphoma, myeloma and solid tumours and use the stem cells as autologous transplants to boost blood production in the same patients undergoing cancer treatment. That led them to question whether peripheral blood stem cells could also be used for allogeneic transplants, and the answer is yes. When growth factors became available that could boost the numbers of peripheral blood stem cells, the possibility of harvesting donor peripheral blood stem cells for allogeneic transplants became a reality in 1993. The first patient, a man with lymphocytic leukemia who was unable to undergo bone marrow transplantation, benefited from the procedure and was lucky enough not to experience graft versus host disease, making the procedure a resounding success. Today, peripheral blood stem cells are routinely used as a source of hematopoietic stem cells for allogeneic transplants, and the growing number of clinical trials is allowing the comparison between matched peripheral blood and matched bone marrow allogeneic transplants. While engraftment is faster and there may be some survival advantage using peripheral blood stem cell transplants, the higher levels of graft versus host disease as compared with bone marrow transplants is a concern. In the case of unrelated (unmatched) donors, studies have not shown any apparent benefits of peripheral blood over bone marrow. However, the practicality of easily harvesting stimulated peripheral blood to purify particular subsets of cells for transplantation is definitely an asset that is worth the continued research evaluating this source of stem cells as a cell therapy for blood diseases.

Umbilical cord blood stem cells

As home to a variety of different types of stem cells, umbilical cord blood holds much promise as an up-and-coming cell therapy. The first cord blood transplant occurred between a brother and sister in 1988 and was used to treat Fanconi's anemia, a genetic condition that tends to lead to myeloid leukemia and bone marrow failure. This transplant was matched but subsequent transplants showed that it was also possible to transplant mismatched cord blood, with less GVHD and the same survival benefit as compared with mismatched bone marrow transplants. The main advantages with cord blood are that it is easy to obtain without risk to the donors, there is less chance of transmitting infectious viruses, and there are no ethical issues associated with its use. Cord blood is also very easy to tissue type and once frozen it can be quickly thawed when it is needed. Today, the practice of using cord blood transplants as a source of hematopoietic stem cells has superseded the use of bone marrow, and saved countless lives for whom no matched bone marrow is available. The collection of cord blood in banks worldwide has facilitated over 20,000 cord blood transplants for treating various blood diseases.

Although high demand versus cord availability is a growing problem, the major limitation with cord blood transplants is good engraftment. Cord blood is known to contain quite a variety of different types of stem cells (hematopoietic, mesenchymal, endothelial, unrestricted somatic, and very small embryonic-like), but the actual numbers of stem cells per cord are still too low to provide optimal engraftment in adult patients. On the laboratory front, scientists are addressing the problem by working with growth factors and signaling pathways that promote self-renewal and expansion of stem cells. In the clinical setting, researchers are boosting the numbers by doing double cord blood transplants, and they are also testing whether different routes of injection or molecules that regulate stem cell homing can help to improve engraftment.

Disease-specific induced pluripotent stem cells (iPS)

The discovery of iPS technology in 2006 was nothing short of revolutionary: its discoverer, Dr. Shinya Yamanaka was awarded the Nobel Prize in medicine (along with Dr. John Gurdon) in 2012. Yamanaka managed to turn back the clock on adult skin cells and reprogram them to a younger, embryonic-like state. The cells are called 'pluripotent' because they are no longer locked into making only one cell type but instead can produce a variety of different cell types.(In Latin, 'pluripotent' means 'very many' and 'having power'.)In the six short years since the technology was first developed, scientists now have methods in place for differentiating iPS cells from skin into a variety of variety of specialized cells, including brain, heart and blood cells.

Despite the excitement and many early experiments showing great promise, scientists are proceeding with extreme caution as there are a number of barriers to overcome prior to safely using iPS-derived cells in patients. Similar to

embryonic stem cell technology, iPS cell technology carries the risk of tumour formation. The original reprogramming method developed by Yamanaka used a mixture of four different viral genes to reprogram adult skin cells. The progress since then has been exponential and now there are safer and more efficient methods for reprogramming virtually any adult cell type. Another approach is to first separate iPS cells from the mature cells they make, and use only the mature cells for therapy. It remains to be seen whether this technique can eliminate the risk of tumour formation. Scientists have also devised a wayto bypass the intermediate iPS state altogether, and instead use the iPS technology in a sideways application to turn one type of adult cell into another type of adult cell, in a process calledtransdifferentiation.

The potential to use iPS cells for the purpose of transplantation is still being vigorously tested in animals but in the meantime, scientists are harnessing the power of iPS technology to create banks of disease-specific iPS cell lines as research tools for modelling diseases and screening candidate drugs.

Gene therapy

What happens in cases where there is a deficiency in the hematopoietic stem cell itself, as for example occurs in people with primary immune deficiencies? In this case, transplanting the patient's own stem cells would be akin to adding insult to injury. Enter gene therapy: the promise of correcting defects at a cellular level to restore normal function. Gene therapy has been used with success to treat immune deficiencies such as SCID but there is still a long way to go before it becomes the standard of care for treating the vast array of blood disorders. Scientists are diligently striving to perfect the process first in mice. They are working out how to stimulate HSCs to grow efficiently and make the corrected genes of interest, and how to develop gene therapy methods that don't cause downstream cancers, such as leukemia.

The combination of gene therapy and iPS technology is being eagerly tested in the laboratory. Skin cells from patients with Fanconi's anemia have been genetically corrected and then reprogrammed into patient-specific iPS cells. It was with much excitement that the scientists found that the corrected iPS cells were able to make disease free, patient-specific cells of the lymphoid and myeloid lineages.This experiment has helped to provide the initial proof that gene corrected disease-specific iPS cells might someday be used as an autologous cell therapy.

One of the most promising experiments to date using gene therapy in combination with iPS technology has shown that it is possible to correct a genetic blood deficiency in mice. Using a humanized mouse model of sickle cell anemia, a disease where the sickle-shaped red blood cells impair the delivery of oxygen to the tissues, researchers corrected the hemoglobin defect in iPS cells and then stimulated the iPS cells to become hematopoietic progenitors. When transplanted into the mice, the corrected hematopoietic progenitors were able to restore normal levels of hemoglobin. Although much work still needs to be done before testing this approach in humans, the field continues to proceed with cautious optimism that the future may include the addition of corrected iPS derived cells as a cell therapy for restoringthe health of patients with blood and other diseases.

Web Resources

Readers may wish to peruse the recommended sites below for more information about blood disorders and the possible applications of stem cells to treat related diseases.

- Leukemia and Lymphoma Society of Canada: http://www.llscanada.org/
- C17 Children's Cancer and Blood Disorders: http://www.c17.ca/
- PubMed Health: http://www.ncbi.nlm.nih.gov/pubmedhealth/
- American Society of Hematology: http://www.hematology.org/Patients/Blood-Disorders.aspx
- Centre for Disease Control and Prevention: http://www.cdc.gov/ncbddd/blooddisorders/index.html
- EuroStemCell: http://www.eurostemcell.org/factsheet/leukaemia-how-can-stem-cells-help

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