Graft Versus Host Disease

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About Graft Versus Host Disease

Background

Commonly referred to as GVHD, graft-versus-host disease is a man-made condition that arises when patients are transplanted with donor tissue that is not identical to their own. Transplants from other individuals are called allogeneic (‘allo’ meaning ‘other’ and ‘geneic’ referring to ‘genes’). GVHD occurs after hematopoietic stem cell or bone marrow transplantation but is most commonly experienced in patients who have received a mismatched graft.

For millions of people with cancer and other blood related disorders, allogeneic hematopoietic stem cell transplants (HSCTs) rescue the patient’s blood system following radiation and chemotherapy treatments. Although this approach is often the only hope for a cure, the development of GVHD in more than 40 per cent of patients is a heavy price to pay and leads to death in 15 per cent of cases.

The occurrence of GVHD after an allogeneic transplant is in no way surprising. The body has built in defenses to protect itself against assaults from foreign entities, the most common being the multitude of bacteria and viruses that try to gain a foothold in the body each day. Modern medicine has made it possible to transplant donor tissue into a patient but the only problem is that immune cells in the donor graft may perceive the host tissue as foreign. You may wonder why immune cells are present in a donor graft? They are among the many types of cells naturally present in bone marrow, and when bone marrow is collected from a donor for an HSCT, fully functional immune cells, called T cells, are mixed in with the other cells in the graft. When the donor T cells mingle with the host tissue, they may see that it is different from the donor tissue and mount an attack, most often on the patient’s skin, gut and liver, in just the same way as they would normally attack foreign bacteria or viruses.

But graft versus host disease is a double-edged sword, with T cells poised in the middle. On one hand, they are ready to attack host tissues but on the other hand they also turn against tumour cells present in the host. This beneficial aspect of graft versus host disease has come to be known as the graft versus tumour (GVT) effect and it is a very important reason to perform allogeneic HSCT in people with cancer. In fact, allogeneic stem cell transplantation in combination with reduced intensity chemotherapy has been successful in decreasing relapse rates in some solid tumours. However, achieving a balance between GVHD and GVT is tricky and there are still many transplant-related deaths. These can be offset somewhat by trying to find the best bone marrow donor-recipient match, but in only 25 per cent of cases does the allogeneic HSCT come from a sibling, with the remainder of patients having to rely on bone marrow or cord blood banks for allogeneic transplants from unrelated donors.
Symptoms, Prevention and Treatment

GVHD can happen very quickly (acute) or develop slowly (chronic) after a longer period of time. Although lumped under the umbrella of ‘graft-versus-host disease’, acute and chronic types are quite distinct. For starters, acute GVHD usually occurs within 100 days of transplant, whereas chronic GVHD tends to develop at least 100 days after the transplant has occurred. Acute GVHD is characterized by inflammation of the tissues, most often manifest as skin rashes, blisters, gastrointestinal complications (diarrhea, vomiting, anorexia, abdominal pain, mucosal ulcers, bleeding of the mucosal) and liver malfunction resulting in drug toxicity, viral infection, sepsis, and iron overload. All this damage is caused mainly by donor T cells but the diseases and damaged tissue also attracts other immune cells to the scene that contribute to more damage and a further influx of immune cells. Once the vicious self-sustaining cycle of damage in acute GVHD is fully underway, it can be challenging to stop.

We know that the symptoms of chronic GVHD are fuelled by the development of autoreactive (self-attacking) T cells in the patient, but how and why these cells develop is still somewhat of a mystery. Autoreactive T cells release interferon gamma, a mediator that results in collagen deposits and tissue scarring. Over the course of the disease, the damage that unfolds begins to look like the patient’s immune system is attacking his/her own blood vessels. The symptoms of chronic GVHD are too numerous to name here but suffice it to say that just about every part of the body can be involved (skin, mouth, eyes, muscles, joints, genitalia, GI tract, liver, lungs, kidneys, heart, bone marrow). The relentless nature of chronic GVHD makes it the number one cause of death unrelated to relapses that occur after hematopoietic stem cell transplantation.

The best way to prevent GVHD is to match the transplanted donor cells as closely as possible to the host or patient cells in a process called tissue typing. Of all the proteins in the body, it is crucial to try to match up the proteins called HLA antigens that are found on the surface of all the cells. The mixture of HLA proteins belonging to each of us is inherited equally from our parents. The closer the HLA match between the donor and host tissues, the less likely the outcome of GVHD for the host (patient). The most perfect match is from an identical twin but these transplants, called syngeneic, are obviously very rare and they don’t provide the benefit of a graft-versus-tumour effect. Consequently their use depends on the type of disease being treated. Another perfect match is when cells are sourced directly from a patient and then later transplanted back. This type of transplant is called autologous and may be used for a cancer patient following very high dose chemotherapy with or without total body irradiation. However not everyone is a candidate for this type of transplant, and furthermore it too does not generate a graft-versus-tumour effect. Full matches can also come from a sibling, but since relatively few patients are fortunate enough to have matched siblings as donors, most patients rely on public or private registries or cord blood stem cell banks for donated cells.

The severity of GVHD also depends on a number of other factors, including age of the patient, source of the graft, the type of conditioning regimen that is used to prepare the patient for HSCT, and the treatments that are used to control GVHD. Typical conditioning regimens are a combination of chemotherapy, radiation and medications that suppress the immune system. The purpose of the conditioning regimen is threefold: residual cancer cells are destroyed by chemotherapy and radiation; other sensitive cells in the patient, for example stem cells, are destroyed by chemotherapy freeing up space in the bone marrow and blood compartments for the donated cells to fully engraft; and immunosuppressant drugs prevent the patient’s immune system from attacking the donor graft.

Although acceptable matches can be found for allogeneic transplants, the degree of mismatch still often precipitates GVHD. The best way to get around this is to remove all the T cells from the donor graft. This ensures that GVHD does not occur, but it also removes the benefit of graft versus tumour effect and this may result in cancer relapse. The shortage of T cells in depleted grafts may also leave the patient without sufficient immune cell function in the short term to fight off infections. Another way to prevent GVHD is to use drugs that suppress the immune system, such as cyclosporine, methotrexate or tacrolimus. If GVHD does occur, then the standard of care is intravenous glucocorticoids (steroids), such as prednisone, that act to suppress the immune system. However, this therapeutic approach is not specific so while the donor T cell attack against the host is suppressed, so too are the normal immune responses in the patient, leaving him/her susceptible to infections that would otherwise not pose any problem. New interventions for treating GVHD being tested in clinical trials include approaches that interfere with specific aspects of triggering the immune system, or agents that affect the microbiome (bacteria and other microbes present in the gut).

Sadly, many transplant recipients develop steroid-resistant GVHD and for them the prognosis is not good. For example, patients who develop acute graft versus host disease that becomes unresponsive to steroids have a very poor
prognosis, with mortality rates of 60% but reaching as high as 90 per cent. Newer therapies are sorely needed for this population and for others who develop life-threatening GVHD.

How can stem cells help?
Currently, there are two different types of stem cells that can be used to mitigate the development of graft versus host disease: umbilical cord blood stem cells and mesenchymal stromal/stem (MSCs) cells.

Umbilical cord blood stem cells

Cord blood is left in the umbilical cord and placenta following the birth of a baby and it contains a number of different types of stem cells, including hematopoietic stem cells (HSCs) and MSCs. The first successful umbilical cord blood transplant occurred between HLA identical siblings to treat Fanconi anemia. The success of the transplant was marked by the restoration of the patient's red blood cells and the absence of graft versus host disease in the recipient, a state that has been maintained to this day. This pivotal transplant paved the way for researchers to test whether allogeneic (donor) cord blood could be used as transplants in unrelated recipients and the positive outcome has turned the practice of discarding umbilical cords into a biological commodity of great value. The advantages of cord blood are numerous. It is easier to obtain (as compared with bone marrow), with no risk to the donor, it can be readily tissue typed and easily stored for future use. For allogeneic stem cell transplantation, the survival rate is the same as compared with bone marrow HSCT but the level of GVHD is less with umbilical cord blood. Today, the creation of over 100 cord blood banks around the world has facilitated over 20,000 cord blood transplants, and the units of cord blood in these banks is over 600,000 and growing. The practice of banking cord blood is providing physicians with the much-needed opportunity to treat patients, in particular racial minorities, for whom matched bone marrow may not be available, with the added post-transplant benefit of less GVHD.

Mesenchymal stromal/stem cells (MSCs)

Mesenchymal stromal/stem cells, or MSCs for short, are under extensive investigation for a number of reasons. They have the ability to differentiate in the laboratory into a variety of different cell types but their possible role in regenerating damaged tissue is complex and appears to be unrelated to this property. However, at present, what makes these cells such an attractive therapy for GVHD and other diseases is their ability to modulate the immune system, inhibit inflammation, stimulate blood vessel formation, repair tissue and help HSC engraftment. One of the most remarkable things about these cells is that even when the HLA proteins between donor and host are mismatched, they don't seem to attract much attention from the patient's immune system and graft rejection rarely occurs. These characteristics have led MSCs to be broadly studied as potential therapeutics in many different diseases.

It has not been so easy for researchers to study exactly how MSCs work. This is partly because they are not present in high numbers in the body but also because it has been difficult to find markers that can tease them out from neighboring cells. However, researchers have learned that MSCs are crucial for producing factors that construct and maintain the niche that nurtures stem cells in the bone marrow. A growing number of early clinical trials for a number of different diseases are demonstrating that MSCs are largely safe, can provide important growth factors and can effectively dampen and regulate the immune system, and as proof the first published success of using MSCs to mitigate GVHD was in a child who had steroid-resistant acute GVHD.

Many tissues in the body contain MSCs. They are most easily collected from bone marrow, fat and umbilical cord, but MSCs from the bone marrow are the best studied. As basic research into the mechanism of action of MSCs continues, many researchers have been using MSCs to treat many different diseases in animals and the success of these preclinical studies are under investigation.

In 2012, Health Canada gave a conditional approval for the use of a mesenchymal cell product, called Prochymal, for the treatment of steroid-resistant and/or immunosuppressant-resistant acute GVHD in pediatric patients. For this application, Prochymal may be used to treat grades C and D acute GVHD that affects any organ or grade B acute GVHD that affects visceral organs, such as the gastrointestinal tract and liver. The final approval awaits the results of clinical studies that are currently underway to validate the therapeutic benefit for patients.
Research and clinical directions

Learning more about MSCs

Although MSCs have made it to the important stage of being tested in clinical trials, the results to date are somewhat confusing and there are discrepancies that challenge the promise of using MSCs for treating GVHD. For example, while European trial results have been favourable, American trials have been contradictory. The earliest American trials demonstrated that MSCs could play a role in treating acute GVHD but more advanced phase III trials have shown little to no benefit. This situation is making researchers reconsider the logistics of MSC transplantation in more depth, specifically to ask whether certain ways of selecting, handling and growing MSCs from lab to lab are affecting the outcome in clinical trials.

In terms of a place to start, it turns out that there are a number of significant differences between the European and American (Prochymal) trials. For starters, not all donors are equal. New research is showing that MSCs must be physiologically activated, or 'licensed' before they are able to regulate the immune system, and perhaps not surprisingly, not everyone is equally capable of doing that. So if MSCs are harvested from donors who are not so good at activating MSCs, then these MSCs when transplanted into patients will not be so good at dampening down the immune response that incites GVHD. One way to address this issue would be to develop screening methods that could distinguish the different types of donors so that only the most active MSCs are used as transplants.

Another issue is the level of expansion (or number of times the cell double) prior to transplanting MSCs. In European trials, MSCs harvested from a single donor are typically expanded into 5 to 10 transplantable doses, whereas in the U.S. Prochymal trial as many as 10,000 doses are expanded from a single donor. This is a huge difference and has researchers wondering whether the function of MSCs might diminish as they are exponentially expanded in the laboratory.

The practice of freezing cells prior to use also needs further investigation. In pre-clinical studies, where the usefulness of MSCs were identified, the cells were harvested, grown for only a very short time and not frozen prior to being transplanted. This approach worked well and the transplanted MSCs were able to modulate the immune system of the diseased mice. It seems logical to question whether freezing cells affects their function, and some studies are now showing that upwards of 20 – 30% of frozen cells are dead upon thawing. Interestingly, the European trials on MSCs did not freeze the cells prior to transplant, but the Prochymal trial did. Clearly, more studies are needed to assess whether frozen versus unfrozen cells are different in their ability to suppress the immune system in recipients.

Finally, researchers are also thinking looking at the continuum of cell products that are given to a patient prior to the MSC transplant, and whether some of these products could promote clearance of MSCs after they are transplanted. As an example, the practice of giving patients platelets prior to allogeneic transplants might in fact sensitize the patient to foreign proteins on the surface of the platelets. If similar proteins are also on the surface of donor MSCs, then it's very likely that the MSCs might be targeted and cleared by the donor's immune system before they have a chance to do any good. Another possible agent that might hamper the ability of MSCs to have an effect in the donor is the type of growing media used to prepare the MSCs for transplant. Some media is made of serum obtained from animals, and the proteins found in animal serum might target the donor MSCs for removal by the patient’s immune system as well.

The ideas described above represent a sampling of the many topics that researchers are looking into to rationalize the different results coming out of clinical trials with MSCs. Going forward, scientists agree that it will be crucial to distinguish the various MSC populations, in terms of their tissue of origin, and the ways in which they work in the laboratory, animal models and patients. This will help researchers to design better clinical trials, taking into account all the known factors that could impact MSC functionality. It will also be important to discover ways to monitor MSCs and to track the clinical outcome in patients who have received MSC transplants. To that end, experts have recommended that a patient registry compiling adverse side effects would be very useful for long term follow-up studies. Although MSCs have burst onto the scene of biomedical research in the past decade, much work at the basic research level is still required before their potential as a therapy for GVHD and other diseases can be fully realized.

The promise of iPS cells

The revolutionary new technology for making induced pluripotent stem cells could someday provide a way to create
patient-matched hematopoietic stem cells for transplantation. The discovery of iPS technology in 2006 was nothing short of revolutionary: its discoverer, Dr. Shinya Yamanaka was awarded the Nobel Prize in Medicine or Physiology (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 in the laboratory 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 and the addition of these genes is not ideal in a clinical context. 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.

A significant amount of work needs to be done before hematopoietic stem cells made from iPS technology can be used on the frontlines for patient care. As a start, researchers are first testing whether human HSCs derived from induced pluripotent stem cells could be used for treating congenital blood disorders, such as anemia and thalassemia, in animal models of disease. These pre-clinical studies are underway but as yet, there has been no demonstration of long-lasting effect. One of the difficulties researchers are coming up against is that the HSCs produced by IPS technology are not functional. There are some who attribute this problem to the lack of a robust niche, or microenvironment, that is able to nurture the developing hematopoietic stem cells and much work is being done to find systems that can better resemble the niche. One research group is even developing a method for growing iPS-derived HSCs in teratomas (benign tumours) in mice because teratomas contain many of the elements of a functional niche that can support HSCs.

The clinical application of cells made from iPS technology is still years away. In the meantime scientists are putting iPS technology to good use. By making banks of disease-specific iPS cell lines, a wonderful new resource has been created for studying disease in a dish and for screening new candidate drugs.

Selected Reading List

- Ringen O, Leblanc K. Best Practice and Research Clinical Haematology. Mesenchymal stem cells for acute and chronic graft versus host disease, tissue toxicity and hemorrhages.