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OBSERVATION

Twisting immune responses for allogeneic stem cell therapy

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Abstract

Stem cell-derived tissues and organs have the potential to change modern clinical science. However, rejection of allogeneic grafts by the host's immune system is an issue which needs to be addressed before embryonic stem cell-derived cells or tissues can be used as medicines. Mismatches in human leukocyte class I antigens and minor histocompatibility antigens are the central factors that are responsible for various graft-versus-host diseases. Traditional strategies usually involve suppressing the whole immune systems with drugs. There are many side effects associated with these methods. Here, we discuss an emerging strategy for manipulating the central immune tolerance by naturally "introducing" donor antigens to a host so a recipient can acquire tolerance specifically to the donor cells or tissues. This strategy has two distinct stages. The first stage restores the thymic function of adult patients with sex steroid inhibitory drugs (LHRH-A), keratinocyte growth factor (KGF), interleukin 7 (IL-7) and FMS-like tyrosine kinase 3 (FLT3). The second stage introduces hematopoietic stem cells and their downstream progenitors to the restored thymus by direct injection. Hematopoietic stem cells are used to introduce donor antigens because they have priority access to the thymus. We also review several clinical cases to explain this new strategy.

Key words: Stem cell therapy; Immune response; Allogeneic grafts

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INTRODUCTION

Ever since stem cells were discovered during the analysis of teratocarcinoma in 1964, stem cell-based therapy has conjured a hope for those who are suffering from previously incurable diseases, particularly degenerative diseases[1,2]. Since embryonic stem cells (ESC) are pluripotent, stem cell-derived cells and tissues are believed to be the best treatment for variety of degenerative diseases by replacing damaged tissues. However, there are still ethical and technical barriers which need to be overcome before these hopes become reality. For example, ESC-driven tumor formation is one technical barrier[3]. Immune rejection caused by foreign antigens expressed on the stem cell graft would be another major hurdle that needs an immediate solution for stem cell therapy[4-6].

Immune rejection concerns are raised when using stem cells that do not exactly match a recipient's immune system - such as existing human embryonic stem cell (hESC) lines that are not derived from the recipient[7]. For example, Wu's group showed that transplanted ESCs

died within about 7 to 10 d in mice with functioning immune systems while they survived and proliferated in immunocompromised mice[5]. They showed that secondary injections of ESCs into the immune-normal mice led to more rapid cell death, suggesting the immune system became more efficient at recognizing and rejecting the second dose of ESCs. It is believe that ESCs express certain surface proteins that trigger the recipient's immune system to attack donor ESCs as they differentiate into more-specialized tissues. Thus the first ESC injection primed the immune system to recognize the foreign molecules, and the immune system responded even more quickly to the second ESC injection. Combination of two antirejection compounds - tacrolimus and sirolimus - allowed the cells to survive for up to 28 d in mice with normal immune systems[4,5]. This is consistent with strategies to prevent T cell activation or effector function by immunosuppression in organ transplantation using pharmacological immunomodulatory agents[8-10].

Several new strategies have been developed to avoid immune rejection of stem cell-derived grafts. These include the use of novel immunosuppressants[11] and of autologous stem cells drawn from somatic cell nuclear transfer (SCNT) and inducible pluripotent stem cell (iPSC) technology[12,13]. However, little is known about the immune response toward these stem cells because of the lack of human clinical trials with these cells. Here, we discuss a new strategy to overcome the hurdle of immune rejection in stem cell therapy of human diseases.

MECHANISMS OF IMMUNE REJECTION

Immune rejection occurs when a transplanted stem cell is not accepted by the body of a transplant recipient. This is expected to happen, because the immune system is able to distinguish a foreign material within the body and try to destroy it, just as it tries to destroy infecting organisms such as bacteria and viruses[14,15]. Allogeneic graft rejection and organ maintenance are the two primary factors, which render donor organs competitive. To be eligible to receive a donor organ, an individual has to pass several compatibility tests[16]. However, rejections may occur even if a patient passes the needed compatibility tests.

Rejections are classified into three major types based on their severities: hyperacute rejection, acute rejection and chronic rejection. Hyperacute rejection happens within short duration after the transplantation process. Preexisting antibodies, which are reactive to the donor tissue, can cause a series of severe systemic inflammatory responses following by blood clotting. Therefore, the transplanted organ must be removed if hyperacute rejection occurs[17-19]. On the other hand, acute rejection and chronic rejection are less dangerous compared to hyperacute rejection. Acute rejection usually occurs within one week after the transplantation because of human leukocyte antigen system (HLA) antigen mismatch. Chronic rejection refers to mismatched minor histocompatibility complex, resulting in long-term rejection of the graft[20]. Since perfect matches between donor and recipient HLA antigens are rare, donor organ recipients often suffer from acute rejection. According to the Organ Procurement Transplant Network (OPTN) national registry in the United States, about 60%-75% of kidney recipients and 50%-60% of liver recipients will experience acute rejection[21-27]. The only available treatment for acute rejection now is either retransplantation or the use of chemotherapeutic immune suppressant like corticosteroids and calcineurin inhibitors. However, immune suppressants will affect the immune system as a whole and lead to immunocompromise complications.

Immune rejection is mediated through both T cell-mediated (direct) and humoral immune (antibodies, indirect) mechanisms. Direct rejections involve the contact between donor antigen presenting cells (APC) and recipient T cells. Antigens on the surface of donor APC can be recognized as foreign particles by recipient T cells through ligation of co-stimulatory molecules. Indirect rejections involve antigens released into the environment, which can be picked up by the recipient APCs and present to the recipient T cells[12,13]. The number of mismatched alleles determines the speed and magnitude of the rejection response. Different mechanisms act against different grafts.

ESCs and their derivatives express human leukocyte class I antigens (MHC I) and minor histocompatibility antigens, both have the potential to trigger host immune rejection[28]. Additionally, ESC may also differentiate into blood cells that express different ABO blood group antigens, which are also immunogenic. Blood group O is a universal donor, which can be selected to avoid rejections caused by ABO blood group antigen differences. In addition, ESCs also express embryo-specific antigens. These embryo-specific antigens, produced only in the embryo stage are treated as foreign particles in a fully developed human body[12,13].

Expressed xenogeneic proteins derived from culture medium may also be a source of immune rejection[29]. An immune response to these antigens may lead to a late graft loss or hastened rejection of subsequent stem cell grafts[30]. This immune rejection can be overcome either by physical immunoprotection of stem cells provided by polymer encapsulation[31-33] or by purification of stem cells before transplantation[34]. Alternative methods to eliminate this potential problem include growing cells in a serum-free medium[35-38].

TWISTING THE IMMUNE RESPONSES

The simplest way to avoid rejection is to use autologous adult stem cells (autologous bone marrow derived mesenchymal stem cells) instead of ESCs which are derived from embryos. Autologous adult stem cells express antigens identical with the hosts' cells and therefore are not subject to the rejections. However, it is difficult to isolate a sufficient amount of adult stem cells[39]. *Ex vivo* expansion and differentiation of adult

stem cells are another major challenge and roadblock for adult stem cell therapy[39]. Purifying adult stem cells from diseased cells (such as autologous bone marrow transplantation) is also technically difficult. Various methods such as SCNT and iPSC technology have been developed to yield sufficient amounts of patient-specific stem cells for cell therapy. However, these methods have not yet been achieved for the status of clinical application.

Manipulating the central self-tolerance pathway is a more immediately available approach for stem cell therapy[28]. There are two types of self-tolerance: central self-tolerance and peripheral self-tolerance[12,13]. Central self-tolerance refers to negative selection of T regulatory cells and T cells within the thymus. Under normal circumstances, T cells differentiated within the thymus do not leave the thymus immediately. Instead, they undergo a process called negative selection, which deletes the host-reactive T cells before releasing them into the circulation. On the other hand, peripheral self-tolerance refers to inappropriate co-stimulation between APCs and T cells which lead to failure to launch proper responses (inflammatory).

Technically, host-donor chimerism can be created to introduce self-tolerance to the donor cells with two steps. First, we can restore thymic function for T-cell selection in host. Second, we need to introduce donor cells into host thymus to delete T-cells that recognize donor antigens[40]. The thymus shrinks during puberty because of increasing production of sex hormones, and its functions are affected[41-44]. Therefore, thymic functions must be restored in order to manipulate self- tolerance in adults. The following strategy can be applied: first, sex steroid inhibitory drug (LHRH-A) can be used in combination with thymic growth factors including KGF, IL-7 and FLT3 to restore adult thymic functions[45]. Second, antigens derived from donor cells must be introduced into the restored thymus[46]. Hematopoietic stem cells (HSCs) can be used to deliver donor cell antigens since they have preferential access to the thymus[46]. However, injecting a large quantity of HSC may trigger serious graft-versus-host diseases, which result in side effects including hemolysis, lost of lymphocytes and possibly damaged tissues because of reactive donor T-cells. Therefore, direct injection is not ideal and an alternative method must be developed to avoid serious graft-versus-host diseases while supplying the HSC steadily to the restored thymus[47,48].

After introducing antigen, a phenomenon called mixed chimerism may occur within the thymus and the donor reactive T cells could be deleted by negative selection[49-52]. Also, T-regulatory cells formed from the thymus may migrate into the circulation and arrest those donor reactive T-cells in the circulation. This immune tolerance has been reported in experiments with mouse skin grafts[53-60].

In animal models, gradually introducing donor cells is a possible approach to create desirable chimeras for thymus restoration. Our previous study on tissue regeneration during mouse pregnancy showed that fetal stem cells repaired maternal skin injury and created fetal-maternal microchimerism[61]. This study suggests that slow release of stem cells that are minimally immunogenic could be used as an alternative method to create chimeras for donor specific immune tolerance. In another study of mouse hematopoietic stem cells, we used intraosseous infusion, a process of injection directly into the marrow of the bone, for introducing HSC into mouse[62]. Intraosseous infusion of HSC allows cells to home to bone marrow more efficiently and avoids circulating large amount of donor cells in recipient blood. This dramatically lessens the chance of immune rejection and may lead to more effective creation of chimerism[61,62].

CLINICAL SUCCESSES FOR MANIPULATING SELF-TOLERANCE

In humans, naturally occurring mixed chimerism self-tolerance has been reported[63]. In one case, a nine-year-old girl who acquired acute fulminant hepatitis from viral infection was given a liver transplant. However, the donor was a 12 year-old boy who died of brain injury having different HLA antigens (A34, 68; B50, 76; DR4, 13) from the girl's (A2, 24; B37, 62; DR7, 9). In addition, their blood types were also different. The girl was type O, RhD-negative but the boy was type O RhD-positive.

The allograft was thought to have a high potential for triggering acute and chronic rejections, which eventually might lead to destruction of the graft. Additionally both of them were cytomegalovirus positive, which may have negative effects on the immunocompromised patient. However, the liver was still transplanted since benefit was determined to be greater than the risk. Standard immunotherapy including tacrolimus and methylprednisolone was given to the girl after the transplantation. Ganciclovir was also given to the girl to try to get rid of the cytomegalovirus. Only 13 d after the transplantation, acute rejection was noted by acute biliary obstruction. The surgical formation of a communication between the common bile duct and the duodenum was performed. The immunosuppressant drugs were given continuously.

The girl suffered from moderate lymphopenia (lymphocyte count, 0.5×10^9 per liter) and anemia until about nine months after the transplantation. Her red blood cell and lymphocyte counts decreased while the white blood cell counts increased, which suggested that her B cells were making antibodies targeting the donor red blood cells resulting in hemolysis. Interestingly, her blood type changed from RhD-negative to RhD-positive, which was the donor's blood type nine months after the transplantation was given. The change showed she developed mix chimerism, which was possibly because of hematopoietic stem cells migrating from the allogeneic graft to the thymus. Therefore the physicians decided to do a series of follow-up studies including

analysis of XY chromosomes on the hematopoietic cells. The result was astonishing, 94% of her T cells, 98% of her B cells and 100% of her natural killer cells had XY chromosomes. The appearance of XY chromosomes on those cells was strong evidence suggesting HSC from the allogenetic graft had successfully migrated into her thymus and proliferated since the female only had XX chromosomes[64]. The surgeons then withdrew the immunosuppressive drug gradually to see if full engraftment could be achieved. The girl remained healthy for the next five years without any sign of rejection. Her liver was functioning normally, and her HLA antigen changed to the donor's type. In this case, the infection with cytomegaloviruses (CMV) in combination with her young age, may have resulted in a more immunocompromised state allowing the full engraftment to be achieved before the graft-versus-host diseases became lethal[63]. In such cases, intraosseous infusion of HSC may be a practical method to create the host-donor tolerance for following organ transplantation or stem cell therapy.

Another successful case was from a recipient of combined kidney and hematopoietic-cell transplants from an HLA-matched donor[65]. There was no rejection or clinical manifestations of graft-versus-host disease without immunosuppressive drugs for more than 24-mo post-transplantation. The blood analyses showed that the patient had persistent mixed chimerism and the function of the kidney allograft was normal.

CLINICAL IMPLICATION

The above case studies suggest that donor-host chimerism can be used to overcome tissue and organ rejection, and slowly introducing donor antigens into host is important to create such chimerism.

Twisting immune responses for allogeneic graft rejection will benefit millions of people worldwide. According to the OPTN (The Organ Procurement and Transplantation Network), there were about 400 000 allogeneic graft recipients in the United States of America alone during the last two decades. In addition, 100 000 patients were queuing on transplant waiting lists for various types of donor organs[26]. Unfortunately, donor organs are competitively allocated, and sometimes it takes years of waiting for an individual to finally be able to receive the donor organ. Sadly, some patients just cannot wait for that long with a dysfunctional organ.

Management of the central self-tolerance pathway provides a possible solution for not only stem cell therapy but all organ transplantation[66]. For example, the use of allogeneic "universal donor" mesenchymal stromal cells (MSCs) may be a great clinical convenience for treatment of autoimmune ailments such as multiple sclerosis[67]. The strategy of manipulating the central self-tolerance pathway may be able to change the appearance of modern clinical science and eventually benefit almost every individual worldwide.

FUTURE DIRECTIONS

The current objective is to reproduce mixed chimerism artificially within an adult with the use of HSCs and immunosuppressive drugs that can restore thymic functions. However, there are still some barriers, which need to be solved before a full engraftment can occur in an adult. Since the thymus atrophies during aging, a sex steroid inhibitory drug (LHRH-A) should be use in combination with thymic growth factors including KGF, IL-7 and FLT3 to restore adult thymic functions[43,68]. After restoration of thymic functions, HSCs need to be introduced into the thymus. Direct injection of HSC and its downstream progenitors (differentiated T cells/T regulatory cells) to the thymus in combination with immunosuppressive drugs (like tacrolimus and methylprednisolone) may allow time to eliminate donor reactive lymphocytes. This also reduces the risk and the severity of hemolysis caused by donor T-cells to the recipient red blood cells or vice versa.

In summary, host-donor chimerism can be used to introduce a specific tolerance for donor tissues. Such a self-tolerance not only makes stem cell therapy one step closer to reality, but also makes organ transplantation available for many patients who cannot find matching donors.

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