Chimerism – a modern strategy for tolerance induction in renal transplantation

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Abstract

Most significant barrier in organ transplantation between genetically different individuals is the immune system that will recognize a transplanted kidney as „non-self" and will reject it. Therefore, one of the most important goals for clinicians, immunologists, researchers working in the field of organ transplantation, is the long-term acceptance of transplanted tissue in the absence of immunosuppression so, tolerance induction.

Over time, it was considered several mechanisms that could induce tolerance. In recent years, it has been proposed and is becoming increasingly studied, a new theory which attempts to clarify graft tolerance. It seems that, the presence of a certain amount of long-lived donor specific cells (hematopoietic or dendritic cells) in the recipient blood flow, plays an important rol in long-term graft acceptance.

Development of new stronger, well targeted immunosuppressive protocols and augmentation of chimeric state even by donor-specific bone marrow infusion, may be useful for long-term graft acceptance in the absence of immunosuppressants, a clinical condition recognized as tolerance.

Key words:chimerism, immunotolerance, microchimerism, rejection, renal transplantation

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Most significant barrier in organ transplantation between genetically different individuals is the immune system that will recognize a transplanted kidney as „non-self" and will reject it. Most of the commonly used immunosuppressive drugs control the rejection process by targeting the immune response nonspecifically but, as lifelong administration is usually required, they often lead to unwanted side-effects including increased susceptibility to infections and development of tumours. Some of these drugs are also associated with nonimmunological complications including an increase in cardiovascular risk factors.

Therefore, one of the most important goals for clinicians, immunologists, researchers working in the field of organ transplantation, is the long-term acceptance of transplanted tissue in the absence of immunosuppression so, tolerance induction. This implies no symptoms of acute or chronic rejection, the absence of circulating donor-specific antibodies and no evidence of subclinical rejection on bioptic specimens. Experimental studies on animal models have showed that long term survival of vascularized grafts can be achieved and more, tolerance induction can effectively prevent the development of chronic rejection [1]. Immunotolerance to alloantigens („non-self") could be induced by exploiting the mechanism that normally maintain the immune system homeostasis and tolerance to „self" antigens. In humans, the occurrence of immunological tolerance has been occasionally reported, in patients who have stopped immunosuppressive treatment because of various reasons (i.e. low compliance, major toxicity, malignancies) and the graft remained still functional.

Among all solid organs that were transplanted, the liver is considered the most privileged in terms of immunology, the most resistant to immunological attack and damage. These observations are not valid for others types of solid organ, kidney in particular, where the harmful effects of acute rejection on graft function and survival are well documented [2-5]. In renal transplantation, it is very difficult for clinical investigators to test different strategies of tolerance induction, because reducing immunosuppression exposes patients to a significant risk of acute rejection occurrence. On the other hand, the situation is complicated by the fact that, at time, there are no certain, specific biomarkers for tolerance assessment. Until such biomarkers are identified and validated, clinical end points of tolerance are limited to a „lack of rejection". In order to address the lack of clinical end points of tolerance, the Immune Tolerance Network (ITN) has established the „ITN Registry of Tolerant Kidney Transplant Patients," a multi-institutional collaboration aiming to identify and study a rare group of renal transplant recipients who have already discontinued immunosuppressive therapy, but have so far not rejected their grafts [6].

Over time, it was considered several mechanisms that could induce tolerance. In recent years, it has been proposed and is becoming increasingly studied, a new theory which attempts to clarify graft tolerance. It seems that, the presence of a certain amount of long-lived donor specific cells (hematopoietic or dendritic cells) in the recipient blood flow, plays an important rol in long-term graft acceptance.

Chimera term defines a person with at least two genetically different cells populations. In humans, this feature is less frequently transmitted congenital but could be acquired by blood transfusion or transplant procedures, either stem cells or solid organ transplantation. Chimerism monitoring has become a routine diagnostic tool in bone marrow transplantation. Are defined three types of chimerism:

1. **Total chimerism** when whole recipient hematopoietic system is destroyed and replaced with donor hematopoietic cells. Unfortunately, this kind of chimerism induction is associated with an incresed risk of graft versus host disease (GVHD).
2. **Mixed chimerism** which assumes donor and recipient hematopoetic cells coexistance. It can be achieved with lethal conditioning regimens.
3. **Microchimerism** that means a low level of donor cells in the recipient blood stream. This situation occurs spontaneously in some patients undergoing solid organ transplantation, due to persistence of donor hematopoetic cells in the allograft which then migrate into de recipient circulatory system. Microchimerism might be one of the ways to achieve engraftment.

In humans, chimerism involvment in tolerance induction has been proven since 1991 when there were reported two cases of patients who had successfully undergone a bone marrow transplantation for acute leukemia and subsequently developed chronic renal failure requiring kidney transplantation. Rennal allografts were harvested from the same donors who have donated hematopoietic stem cells. The transplanted kidneys were accepted by the hosts in the absence of the immunosuppresive therapy [7-10].

However, routine use of the chimerism concept in

clinical practice in the field of solid organ transplantation, was ruled out because of high toxicity of conditioning regimens and because of increased risk of GVHD following myeloablative therapy. Subsequently, experimental studies on mice have shown that tolerance could be induced using also non-myeloablative conditioning regimens, leading mixed chimerism [11-14]. Later, non-myeloablative protocols have been proved safe and effective in humans bone marrow transplanation forhematological malignancies [15-16]. The first attempts to combined transplant, bone marrow and kidney, with non-myeloablative conditioning were conducted in patients with chronic renal failure secondary to multiple myeloma. Conventionally, in these kinds of patients, bone marrow transplantation is contraindicated due to renal failure and kidney transplantation is contraindicated because of hematological malignancy [17-19]. Of nine patients HLA-matched with donors, treated in this way, only one developed renal rejection, quickly overcome with temporary immunosuppressive therapy. The longest period of normal functioning renal graft without rejection signs in the absence of immunosuppression, was 12 years. Regarding multiple myeloma, only three patients developed recurrent disease, one of them dying seven years later due to progressive multiple myeloma.

Other studies have shown that mixed chimerism and graft tolerance could be induced using costimulatory blocking agents [20] or T cell–depleting antibodies [21], strategies that are more clinically acceptable.

On the other hand, a condition of very low level of donor chimerism (microchimerism) occurs spontaneously in a fraction of organ transplant patients as a result of persisting donor hematopoietic cells that have migrated from the transplanted organ into the host.

Animal models experiments have shown that microchimerism contributes to allograft acceptance. In a rat model, depletion of intragraft donor leukocytes at the time of heart transplantation was associated with acute graft rejection, whereas untreated animals developed long-term graft survival [22]. To facilitate the development of microchimerism, various strategies have been tested both in experimental animals and in transplanted patients by infusion of different donor cells (bone marrow / mature leukocytes such as splenocytes / dendritic cells / peripheral blood mononuclear cells) [23-28]. Despite the high number of studies, which type of donor cells and whether mature or immature donor cells should be infused to achieve the best tolerogenic result have not been clarified yet.

Peripheral blood microchimerism in renal transplant recipients is still a subject for debate. Fourtounas et al., in a small number of patients study, have not found peripheral blood microchimerism but they have found microchimerism in urine in almost half of patients, especially in the first week post-transplant [29].

Microchimerism detection is highly dependent by the sensitivity of the tests used. Polymerase Chain Reaction (PCR) as a rapid method for amplification of microsatellites and minisatellites regions of the human genome has provided a powerful and reliably tool for the chimerism study. PCR method allows detection of a minor donor cells population among recipient cells. Generally, the microsatellites (STR - short tandem repeats) analysis is simpler, more accessible than minisatellites (VNTR - variable number of tandem repeats) analysis.

Using a nested-PCR method with a sensitivity up to 1:1,000,000, a group of scientists from Tehran, have been detected the peripheral blood microchimerism in 21.9% of renal unrelated living donor transplanted patients. Microchimerism was not detected in recipients from cadaveric donors [30]. Similar results has also obtained a group of researchers from Sao Paolo, Brazil. Four of five living donor recipients have developed blood microchimerism and all of them had a better outcome[31].

Other studies have shown that, with routine immunosuppressive protocols, a better HLA antigens/alelles matching, especially HLA class II (HLA-DRB1), facilitates microchimerism induction [32].

In conclusion, development of new stronger, well targeted immunosuppressive protocols (including conditioning) and augmentation of chimeric state even by donor-specific bone marrow infusion, especially in completely mismatched HLA-DRB renal allograft recipients, may be useful for long-term graft acceptance and improved quality of life in the absence of immunosuppressants, a clinical condition recognized as tolerance.

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References

1 Madsen JC, Yamada K, Allan JS et al.: *Transplantation tolerance prevents cardiac allograft vasculopathy in major histocompatibility complex class I-disparate miniature swine.* Transplantation 1998; 65(3): 304-13.

2 Opelz G, Döhler B: *Influence of time of rejection on long-term graft survival in renal transplantation.* Transplantation. 2008; 85(5):661–66.

3 Leggat JE, Ojo AO, Leichtman AB et al.: *Long-term renal allograft survival: prognostic implication of the timing of acute rejection episodes.* Transplantation. 1997;63(9):1268–72.

4 Heilman RL, Nijim S, Chakkera HA, Devarapalli Y et al.: *Impact of Acute Rejection on Kidney Allograft Outcomes in Recipients on Rapid Steroid Withdrawal. Clinical Study.* Journal of Transplantation. 2011; doi:10.1155/2011/583981, ID articol 583981, 8 pag

5 McCauley J: *Long-Term Graft Survival In Kidney Transplant Recipients.* Medscape Transplantation. 2004;5(1)

6 Matthews JB.: *New Renal Transplant Registry Targets Immune Tolerance.* Medscape Transplantation. 2004;5(2) © 2004 Medscape

7 Sayegh MH, Fine NA, Smith JL, Rennke HG, Milford EL, Tilney NL: *Immunologic tolerance to renal allografts after bone marrow transplants from the same donors.* Ann Intern Med. 1991; 114(11):954–55.

8 Helg C, Chapuis B, Bolle J-F et al.: *Renal transplantation without immunosuppression in a host with tolerance induced by allogeneic bone marrow transplantation.* Transplantation. 1994; 58(12):1420–22.

9 Jacobsen N, Taaning E, Ladefoged J, Kristensen JK, Pedersen FK: *Tolerance to an HLA-B,DR disparate kidney allograft after bone- marrow transplantation from same donor.* Lancet. 1994;343(8900):800.

10 Dey B, Sykes M, Spitzer TR: *Outcomes of recipients of both bone marrow and solid organ transplants. A review.* Medicine (Baltimore). 1998; 77(5):355–69.

11 Tomita Y, Khan A, Sykes M: *Role of intrathymic clonal deletion and peripheral anergy in transplantation tolerance induced by bone marrow transplantation in mice conditioned with a nonmyeloablative regimen.* J Immunol. 1994; 153(3): 1087–98.

12 Tomita Y, Sachs DH, Khan A, Sykes M: *Additional mAb injections can replace thymic irradiation to allow induction of mixed chimerism and tolerance in mice receiving bone marrow transplantation after conditioning with anti-T cell mAbs and 3 Gy whole body irradiation.* Transplantation. 1996; 61(3):469–77.

13 Khan A, Tomita Y, Sykes M: *Thymic dependence of loss of tolerance in mixed allogeneic bone marrow chimeras after depletion of donor antigen. Peripheral mechanisms do not contribute to maintenance of tolerance.* Transplantation. 1996; 62(3):380–87.

14 Manilay JO, Pearson DA, Sergio JJ, Swenson KG, Sykes M: *Intrathymic deletion of alloreactive T cells in mixed bone marrow chimeras prepared with a nonmyeloablative conditioning regimen.* Transplantation. 1998; 66(1):96–102.

15 Spitzer TR, MCafee S, Sackstein R et al.: *The intentional induction of mixed chimerism and achievement of anti-tumor responses following non-myeloablative conditioning therapy and HLA-matched and mismatched donor bone marrow transplantation for refractory hematologic malignancies.* Biol Blood Marrow Transplant. 2000; 6(3A):309–20.

16 Dey BR, MCafee S, Colby C et al.: *Impact of prophylactic donor leukocyte infusions on mixed chimerism, graft-vs-host disease and anti-tumor response in patients with advanced hematologic malignancies treated with nonmyeloablative conditioning and allogeneic bone marrow transplantation.* Biol Blood Marrow Transplant. 2003; 9(5):320–29.

17 Spitzer TR, Delmonico F, Tolkoff-Rubin N et al.: *Combined histocompatibility leukocyte antigen-matched donor bone marrow and renal transplantation for multiple myeloma with end stage renal disease: the induction of allograft tolerance through mixed lymphohematopoietic chimerism.* Transplantation. 1999; 68(4):480–84.

18 Buhler LH, Spitzer TR, Sykes M et al.: *Induction of kidney allograft tolerance after transient lymphohematopoietic chimerism in patients with multiple myeloma and end-stage renal disease.* Transplantation. 2002; 74(10):1405–09.

19 Fudaba Y, Spitzer TR, Shaffer J et al.: *Myeloma responses and tolerance following combined kidney and nonmyeloablative marrow transplantation: in vivo and in vitro analyses.* Am J Transplant. 2006; 6(9):2121–33.

20 Wekerle T, Kurtz J, Ito H et al.: *Allogeneic bone marrow transplantation with co-stimulatory blockade induces macrochimerism and tolerance without cytoreductive host treatment.* Nat Med. 2000; 6(4): 464–69.

21 Fuchimoto Y, Huang CA, Yamada K, Shimizu A, Kitamura H, Colvin RB, Ferrara V, Murphy MC, Sykes M, White-Scharf M, Neville DMJr, Sachs DH: *Mixed chimerism and tolerance without whole body irradiation in a large animal model.* J Clin Invest. 2000; 105(12): 1779–89.

22 Ko S, Deiwick A, Jäger MD, et al.: *The functional relevance of passenger leukocytes and microchimerism for heart allograft acceptance in the rat.* Nat Med. 1999; 5(11): 1292–97.

23 Umemura A, Monaco AP, Maki T: *Donor MHC class II antigen is essential for induction of transplantation tolerance by bone marrow cells.* J Immunol. 2000; 164(9): 4452–57.

24 Mathew JM, Garcia-Morales R, Fuller L, et al.: *Donor bone marrow–derived chimeric cells present in renal transplant recipients infused with donor marrow. I. Potent regulators of recipient antidonor immune responses.* Transplantation. 2000; 70(12): 1675–82.

25 Yang L, DuTemple B, Khan Q, Zhang L: *Mechanisms of long-term donor-specific allograft survival induced by pretransplant infusion of lymphocytes.* Blood. 1998; 91(1): 324–30.

26 Goldstein DR, Chang T, Sweeney SD, et al.: *Enhanced allograft survival induced by posttransplant donor spleen cell infusion occurs via a mechanism that is distinct from the mechanism of enhancement by donor bone marrow.* Transplantation. 2000; 69(5): 1020–22.

27 Lu L, Li W, Zhong C, et al: *Increased apoptosis of immunoreactive host cells and augmented donor leukocyte chimerism, not sustained inhibition of B7 molecule expression are associated with prolonged cardiac allograft survival in mice preconditioned with immature donor dendritic cells plus anti-CD40L mAb.* Transplantation. 1999; 68(6): 747–57.

28 Noris M, Azzollini N, Mister M, et al: *Peripheral donor leukocytes prolong survival of rat renal allografts.* Kidney Int. 1999; 56(3): 1101–12.
29 Fourtounas C, Spzridonidis A, Dousdampanis P, et al.: *Microchimerism in peripheral blood and urine in renal transplant recipients: preliminary results.* Transplant Proc. 2008; 40(10): 3434-36.
30 Saraji A, Pourmand G, Mehrsai A et al.: *Microchimerism and Renal Transplantation: Doubt Still Persists.* Transplant Proc. 2007; 39(4): 948-50.
31 Crispim JC d. O, Wastowski IJ, Faggioni LPC, et al.: *Microchimerism Evaluation in Recipients of Living-Related or Unrelated Deceased Allograft Renal Transplants.* Transplant Proc. 2006; 38(9): 2828-30.
32 Tajik N, Singal D, Pourmand G, et al.: *Prospective study of microchimerism in renal allograft recipients: association between HLA-DR matching, microchimerism and acute rejection.* Clin Transplant. 2001; 15(3): 192-98.

Rezumat

Obstacolul major în transplantul de organe între persoane diferite genetic îl reprezintă sistemul imun care va recunoaşte un rinichi transplantat ca „non-self" şi îl va respinge. De aceea, unul dintre cele mai importante deziderate pentru imunologi, clinicieni, cercetători care lucrează în domeniul transplantului de organe este acceptarea pe termen lung a ţesuturilor transplantate în absenţa imunosupresiei, adica inducerea toleranţei.

De-a lungul timpului s-au luat în considerare mai multe mecanisme care ar putea induce toleranţa. În ultimii ani, a fost propusă şi este din ce în ce mai mult studiată o noua teorie care încearcă să elucideze toleranţa grefelor. Se pare că prezenţa în sângele primitorului a unei anume cantităţi de celule cu durată de viaţă lungă provenite de la donator (celule hematopoietice sau celule dendritice) are un rol important în determinarea acceptarii grefei pe termen lung.

Dezvoltarea unor noi protocoale imunosupresoare, intensive şi bine ţintite, şi augmentarea statusului chimeric chiar prin infuzie de limfocite donor-specifice, poate induce acceptarea grefei pe termen lung în absenţa imunosupresoarelor, condiţie clinică recunoscută ca toleranţă.

Cuvinte cheie: chimerism, imunotoleranţă, microchimerism, rejet, transplant renal