transplantation
india

MEDAWAR MOUSE VISITS AHMEDABAD!

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ABBREVIATIONS

BM : Bone marrow
DST : Donor specific transfusion
GVHD : Graft versus host disease
HSC : Hematopoietic stem cells
HVG : Host versus graft
LCM : Lymphocytotoxicity cross-match
MHC : Major histocompatibility complex
BMCs : Bone marrow cells
PBSC : Peripheral blood stem cells

Modern transplantation immunology has its origin in experimental work of Peter Medawar in 1944 in which he demonstrated that skin allograft rejection was host versus graft (HVG) response [1]. Cell mediated immunity as mechanism of rejection was later defined by Mitchison [2]. The term major histocompatibility complex (MHC) was introduced by Gorer, Lyman and Snell for the genetic locus that encodes antigens associated with allograft rejection, tumor surveillance and other expressions of cell-mediated immunity [3]. Zinkernagel and Doherty demonstrated MHC-restricted mechanisms of T-cell recognition and response to antigens, viruses and other intracellular micro-organisms [4]. The origin of tolerance story dates back to experiments inspired by Owen's description of red blood cell chimerism in freemartin cattle [5]. Billingham, Brent and Medawar recognized the significance of this observation and designed a tolerance model in which allogeneic spleen and bone marrow cells (BMCs) were grafted and induced tolerance in incompletely developed immune system of neonatal mouse recipient [6]. In this model tolerance was extended to donor strain skin allograft. This model had analogy to successful BM transplantation in humans where host cytoablation was necessary to make immune system equivalent to that of neonatal mouse.

Medawar's seminal experimental work which later on became known as "classical tolerance" raised expectations amongst the tolerance researchers that a breakthrough in transplantation tolerance was imminent. These expectations turned out to be unrealistic because the human transplant biology was much different than that of the mouse and it was therefore not very easy to have safe conditioning regimes to allow stem cell grafting across MHC barriers. Even understanding of the mechanisms of rejection process was in very preliminary state and two-drug immunosuppression (with prednisolone and Azathioprine) was ineffective to prevent allograft rejection in approximately 30 % recipients. Presence of associated infections clouded the understanding of immunologic mechanisms. It was not until the arrival of cyclosporine (CsA) as an immunosuppressant and also clear and better understanding of direct/ indirect and other pathways of rejection well understood, that tolerance research was drawn in its right perspective.

In seventies the most exciting immunologic adventures in area of tolerance was restricted to donor specific and third party blood transfusions. It was Gerald Opelz and John Dossetor who drew the attention of the world to the fact that renal allograft recipients who received more number of donor-specific and/ or third party blood transfusions encountered lesser number and intensity of steroid resistant acute rejection episodes and also had better allograft function. This phenomenon which was clinically apparent was not well understood at molecular level of transplant immunology. Paul Terasaki kept on suggesting at different meetings that such benefit could only be possible if allo-specific T-cell clones are activated and exhausted, however this was not a universally

accepted explanation of this benefit. The fear of hypersensitization (which regularly occurred in about 30 % recipients), was an overwhelming disadvantage of this immunological manipulation. CsA arrival very quickly pushed the donor-specific transfusions (DST) in to oblivion only to be rediscovered by a selected group of tolerance researchers in late 90's as a means to induce proliferation of donor-specific clones so that they could easily be deleted. The proliferating clones have an unusually large quantitative requirement of interleukin-2 cytokine and deprived clones undergo apoptotic deletion. Looking back, Terasaki's prophecy was intuitively in the right direction.

My experience with DSTs and early use of anti-thymocyte globulin at McMaster University, Canada, sensitized my thinking process which will lead to the pursuit of the ultimate, "transplantation without drugs"! In earlier days of transplantation at the Institute of Kidney Diseases and Research Centre (IKDRC), I used cytokine mobilized DSTs in living-related donor renal allograft recipients and I have one of the longest survivor; 9 years post-transplant, with full-house MHC- mismatched donor who is on minimum immunosuppression! These were anecdotal stories which happened as pleasant accidents and could not be reproduced regularly.

It was in January 1996, when IKDRC hosted the 8th annual meeting of Indian Society of Organ Transplantation at Ahmedabad. Stalwarts of solid organ transplantation like Prof. Thomas Starzl and Sir Roy Calne introduced to the Indian transplanters the concept of classical Medawarian tolerance in this meeting. This was the turning point of my thinking process at conceptual level and I was convinced that the challenge of time was to validify Medawarian concept in clinical transplantation. More than fifty years have passed after this work and we were no where near to achieving tolerance in clinic. There were numerous reports and experiments generated during these 50 years and the most convincing concept was induction of mixed hematopoietic chimerism through allo-stem cell transplantation as an adjuvant to solid organ transplantation- in our case it was of course the kidney! One of the most interesting corollaries to this non-productive exercise was the inability of stem cell transplanters and solid organ transplanters to share a common platform; instead they were too busy in pursuing their own objectives! We knew it was not easy to bring these two fields together or else it would have been attained by some one long back!

We decided to have our department of stem cell transplantation at the institute so as to subscribe to our common goal of achieving chimerism induced tolerance in clinic through stem cell grafting. It took us approximately two years to get this infrastructure organized and convince other services about their active participation towards a common goal! The objective was clear, plans were made, lab and infrastructure made ready, and on 1st September, 1998 we performed our first allo-stem cell transplantation in living-related donor recipient.

The fear of graft versus host disease haunted us for a while just to find out that we would not be facing this problem at all! We wanted a very safe protocol and our first 27 recipients were administered high dose of hematopoietic stem cell transplantation (HSCT) ($8 \pm 3 \times 10^8$ nucleated cells /kg BW of recipient) only. We reviewed our experience in December 1998 to find out the benefits of this protocol. We observed lesser incidences of acute rejection episodes, CMV infections and allografts survived with two drugs instead of three! The preliminary data was presented at the 6th Congress of Asian Society of Transplantation in Singapore in September, 1999, with Sir Peter Morris presiding [7]. This presentation generated considerable discussion amongst the transplanters. Terasaki in particular made very favorable comments about our work. At the concluding session, Sir Roy Calne described this work as one of the outstanding work presented at this meeting. This work was peer reviewed and published in transplantation proceedings. I returned from the meeting encouraged, stimulated and determined to further modify this model and make it more effective. We came across Gorzinsky's work from Toronto, about using the portal route to administer stem cells. We then modified our protocol and developed our technique of portal infusion of peripheral blood stem cells (PBSCs) through a simple procedure using the omental vein. We implemented this protocol in 234 patients and increased the HSC dose from 10 to15 x10 ^{8} cells/ kg BW and found superior results with this group. I presented this work at the 18th International Congress of Transplantation Society in Rome in August, 2000 [8, 9]. Eminent workers in the field took a note of this presentation seriously. Yet, the goal of robust tolerance evaded us. After reviewing the work of Remuzzi, Posselt and Kriesel we further modified our protocol by adding inoculation of donor bone marrow (BM) derived cells initially, and later on renal tissue in to recipient thymus.

At this juncture we realized about several problems facing us; namely: maximum allo-resistance offered to stem cells derived from a subset of recipient HSCs residing in BM popularly known as cobblestone like colony forming stem cells. Experiments indicated that myelo/ immunosuppressive drugs at their best could not delete or abrogate the function of stem cells. Radiation, Busulfan or Treosulphan were the only tools to effectively abrogate allo-resistance of this group of stem cells. At this point, Slavin's prophecy about the importance of irradiation in achieving grafting in adult immune system was coming to see the light of the day. So, we added 2 DSTs and low intensity conditioning. The first step was to infuse 2 DSTs at intervals of 2 days to stimulate proliferation of donor-specific T-cell clones; followed by series of steps to delete proliferated and proliferating donor-specific alloreactive T-cell clones. We have very soon replaced this with donor leucocyte infusions. This was believed to create "space" in thymus, BM and lymph nodes. The conditioning regime was initiated with fractionated (4 doses on alternate days) low dose (400 cGy which is now increased to 500 cGy) target-specific (abdominal and inguinal lymph nodes, BM of vertebral bodies and part of hip bones) irradiation. Then we administered polyclonal anti-T-cell antibody (rabbit), 1.5 mg/kg BW along with cyclophosphamide, 20 mg/kg BW. Cyclophosphamide was used to create "space" in thymus by deletion of allo-resistive host antigen presenting cells in thymus. Cyclosporine, 3 mg/kg BW was added to protect chimerism and prevent GVHD. Within 24 hours of achieving deletional target of host CD4+/ CD8+ count to less than 10 % of baseline, unmodified donor BM stem cells (400 ml) were infused (60 ml in to iliac crest BM, 200 ml in to portal circulation and 140 ml in periphery). It was supplemented by infusion of 2 cytokine stimulated PBSCs at intervals of 2 to 3 days (Cobe Spectra version 7 and Hemonetics-MCS3p, USA). We aimed at infusing at least 20 x 10^8 cells/ kg BW. Renal transplantation was performed about 1 week after the final HSC infusion following negative lymphocytotoxicity cross matching (LCM). All patients who developed donor-specific cytotoxic antibodies were treated with cyclosporine and cyclophosphamide. In case of high positivity intravenous gamma globulins, splenic irradiation and plasmapheresis were also used. Transplantation was followed as soon as negativity was achieved.

Out of the last group of 110 patients with mean follow-up of 300 days, all patients have adequately functioning grafts with actuarial 1 year graft and patient survival of 100 %! We had 3.6 % steroid responsive acute rejection episodes (type 1A according to modified Banff classification). No CMV infection or any other infective episode has been observed. All of them are on prednisolone, 0.2 mg/kg BW and CsA, 2 mg/kg BW/ day for the first 6 months post-transplant, and low dose CsA monotherapy 9 months post-transplant. We have been able to demonstrate mixed lymphohematopoietic chimerism (0.8 ± 0.2 %) in peripheral blood in all recipients with gender mismatched donors. We have an interesting observation that natural suppressor cells steadily migrate to marrow by the end of one year post-transplantation. This could represent natural suppressor T-cell chimerism and may become the laboratory marker of clinical tolerance.

We believe that Medawarian concept is soon to be validified in "Ahmedabad high dose- deletion- chimerism" tolerance model.

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