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RELATIONSHIP BETWEEN CHIMERISM AND TOLERANCE IN RENAL TRANSPLANTATION

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ABBREVIATIONS

ALL	acute lymphatic leukemia
BM	bone marrow
DST	donor specific transfusion
HSCs	hematopoietic stem cells
MHC	major histocompatibility complex
APCs	antigen presenting cells
BMT	bone marrow transplantation
GVHD	graft versus host disease
HSCT	hematopoietic stem cell transplantation
TCD	T-cell depletion

The ultimate goal in human transplantation is donor-specific tolerance, which is long-term acceptance of an organ/ tissue allograft without using any immunosuppressive regime. Mechanisms for tolerance induction can be divided in to two major categories: central and peripheral. Central tolerance is induced by thymic deletional mechanism. Immature T-cells migrate through thymus where they come in contact with endogenous peptides bound to major histo-compatible complex (MHC) molecules expressed on antigen presenting cells (APCs). T-cells with high affinity interactions with self-peptides are eliminated (negative selection), whereas those with low/ intermediate affinities are positively selected and released in to periphery where they provide immune surveillance. Peripheral tolerance occurs when mature T-cells escape thymic tolerance and come in contact with antigen in the periphery in such a way that T-cells are either deleted or rendered anergic. Central tolerance is more stable than peripheral [1]. (Fig 1)

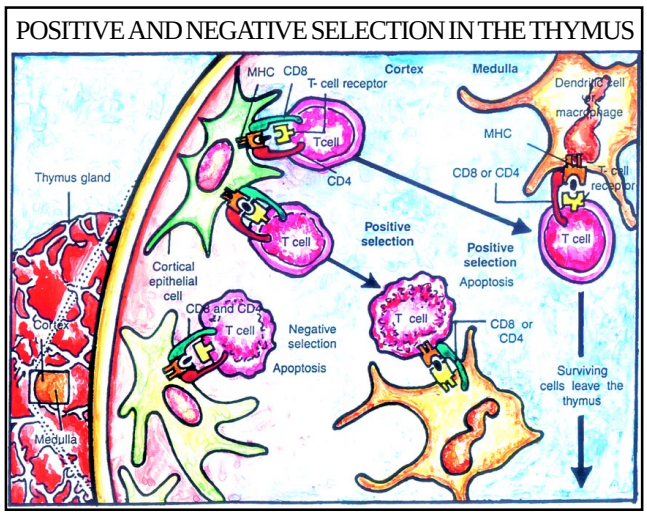

Figure 1 Antigen presenting cell presents MHC molecule to naïve T-cell receptor. T-cell with high affinity for antigen gets deleted by negative selection process. T-cells with low affinity for the antigen escape to the periphery through the process of positive selection.

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MIXED ALLOGENEIC CHIMERISM AND TOLERANCE INDUCTION

Tolerance to allogeneic (non-self) antigens can be induced experimentally by hematopoietic stem cell chimerism [2]. Chimerism is defined as coexistence of two genetically different bone marrow derived cells in one individual. Association between hematopoietic chimerism and tolerance was observed for the first time by Owen in 1945 in freemartin cattle twin sharing a common placenta, who exhibited erythrocytic chimerism [3]. Billingham and Brent observed that skin grafts between dizygotic cattle twins survived, whereas those from third party were rejected [4] (Fig 2).

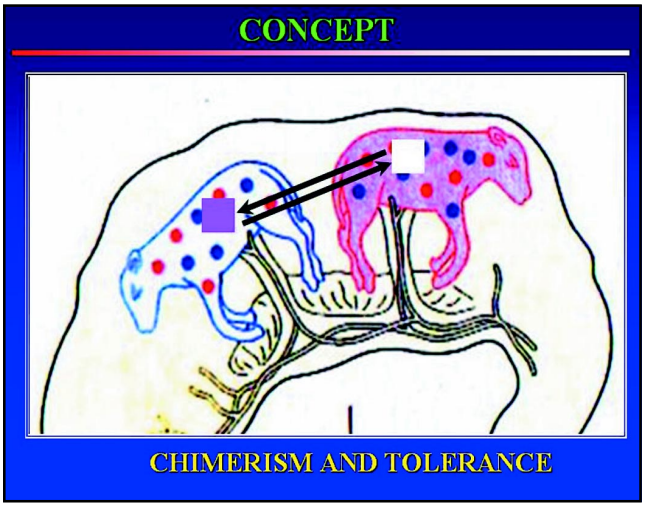

Figure 2 R.D. Owen's model of freemartin cattle twin in which he observed sharing of each other's RBCs (erythrocytic chimerism) long after separation of placenta.

They further carried out studies in neonatal mouse model and injected adult spleen cells in a fetal mouse who subsequently accepted skin grafts from donor mouse, but rejected third strain grafts [5, 6]. (Fig. 3)

Fetal model did not require any conditioning regime. Main, Prehn and later Slavin proved that irradiation was required for adult mice to achieve the same result [7, 8].

About 25 years ago, Monaco identified the potential of tolerance induction in donor bone marrow (BM) [9]. Subsequently BM transplanters have achieved fully allogeneic chimerism by lethal conditioning followed by allogeneic BM infusion. However, full allogeneic chimerism results in to immune incompetence towards primary immune responses [10]. To overcome this limitation, Ildstad and Sachs

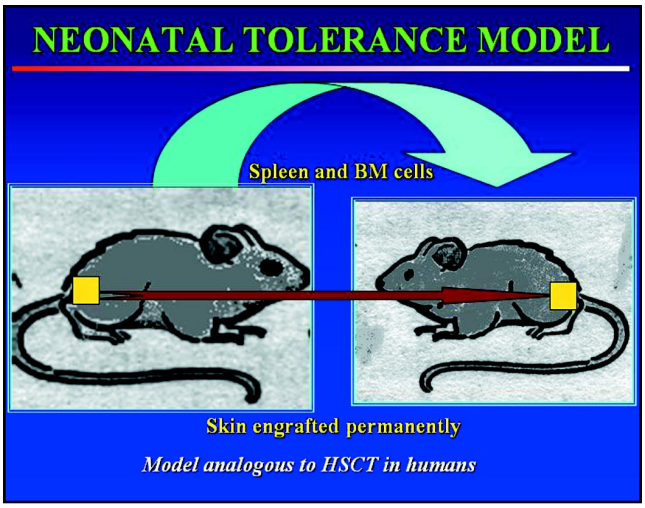

Figure 3 Medawar model showing neonatal tolerance in skin grafts following splenic cell infusion

first described the generation of mixed allogeneic chimerism by transplanting T-cell-depleted (TCD) murine donor and recipient marrow in to a single mouse conditioned by full ablation. This led to long term survival of recipient and donor-type skin grafts with preservation of recipient's immunity to third party antigens [11].

In liver, kidney and heart allografts long term persistence of donor derived dendritic/ hematopoietic stem cells (HSCs) has led to a theory that there is a causal relationship between long term allograft acceptance, chimerism and tolerance [12]. However, the relationship between chimerism and tolerance has not been established. In vascularized organ allograft following induction of lymphohematopoietic chimerism by bone marrow transplantation (BMT), tolerance persists even if the organ allograft is removed [13, 14]. However in case of grafts maintained on immunosuppression without BMT, tolerance disappears with graft removal [15]. This has made some investigators like Wood, Sachs and Fuchimoto believe that it is the grafted organ from which escaped cells maintain chimerism and are not rejected due to immunosuppressive drugs [16, 17]. Persistence of chimerism has not been thought to be essential for tolerance in experiments on swine.

Gammie et al carried out an elegant experiment in rat model to determine the effect of mixed hematopoietic chimerism on chronic rejection in a tracheal transplant model [18]. They observed sustained stable chimerism through all lineages ranging from 58 % to 88 % for more than 20 weeks. Tracheal transplantation from the same donor BM strain in to recipient

omentum (heterotopic) 28 days after BMT was carried out. It survived without immunosuppression 150 days after transplantation. Histologic findings were graded based on luminal obstruction and inflammation. Their data confirmed that HSCs were responsible for preventing chronic rejection in mixed chimeras. Irradiation or tacrolimus administration in controls did not prevent the chronic changes.

Role of HSC in tolerance

Dendritic cells are derived from hematopoietic precursors of myeloid/ lymphoid lineage and some of them home to thymus where they serve as APCs and participate in thymic education [1]. Tolerance is induced only to those antigens introduced to the recipient by hematopoietic cell precursors that engraft or seed in to hematopoietic reservoirs like BM. However hematopoietic precursors can fail to graft and if engrafted, there is a very high risk of graft versus host disease (GVHD) with increasing genetic disparity between donor and recipient.

Donor graft composition

T-cell depletion is not required if there is no/low genetic disparity between recipient and host and thereby maintaining immune competence of the host. However stringent T-cell depletion is required with disparity to prevent GVHD. Yet pure HSC population can be grafted in the host by using appropriate conditioning [19].

Graft rejection can occur in presence of lymphoid chimerism hence it is not necessarily indicative of stem cell engraftment. Homing of donor BM derived APCs to thymus is essential for induction of central tolerance. Organ graft acceptance has been documented in absence of stable (peripheral) chimerism [16].

Stem cell depletion

Central tolerance induction requires depletion/ displacement of recipient stem cells and overcoming host immune resistance. Increasing genetic disparity between host and recipient makes engraftment difficult. Recipient stem cell depletion has been studied in syngeneic animal models by using irradiation, chemotherapeutic agents like busulfan, infusion of high dose autologous HSCs or allogeneic T-cells, administration of radioactively tagged stem cell reactive antibodies and cytokine mobilization of stem cells in to periphery. Stewart, Sykes and Fuchimoto have proved in animal models that engraftment can be achieved using large doses of stem cells; and making "space" in BM is not required [20-22]. Perhaps veto effect helps in engraftment. Amount of chimerism established is related to the extent of stem cell depletion. Tolerance induction can be achieved with very low dose of busulfan at minimal levels of chimerism.

Immunosuppression and tolerance

Central tolerance related to donor HSC chimerism can only be achieved if immunocompetent lymphocytes in periphery are first eliminated or inactivated to circumvent initial immunological rejection of stem cell graft. T-cell depleting monoclonal antibodies have been found to be superior to chemotherapeutic agents like fludaribine or cyclophosphamide by being very specific to T-cell elimination rendering them safe, and since they eliminate both recipient and donor T-cells, there are minimal / no chances of GVHD. However they have a very short half-life.

Strategies for tolerance induction

Slavin et al developed a partial conditioning method for tolerance induction consisting of low dose irradiation to only lymphoid tissues, immunization with donor BM or peripheral blood cells to stimulate donor-specific alloreactive T-cells and subsequent treatment with cyclophosphamide to eliminate these alloreactive T- cells. A final second infusion of T-cell depleted (TCD) hematopoietic donor cells was required to induce more stable chimerism and donor-specific tolerance to skin grafts without GVHD [23]. In this model, mixed chimerism was accomplished with maintained third party immune reactivity by partial conditioning only when donor and host T-cells were balanced against each other.

Sykes et al have developed a BMT strategy that achieves allo-engraftment without toxic or myelosuppressive host conditioning in mouse model where B6 mice received depleting anti-CD 4, anti-CD 8 monoclonal antibodies, local thymic irradiation and a high dose (174.3×10^6) of MHC mismatched BM cells divided over days 0 to 4. Permanent, multilineage mixed chimerism and donor-specific skin graft tolerance were observed in most animals. Large number of donor class II cells were observed in their thymuses and their presence was associated with intrathymic deletion of donor-reactive host thymocytes [22]. Thymic chimerism of donor BM derived APC may be one of the important factors in sustaining donor-specific tolerance. With appropriate conditioning donor APCs will be able to home to reside in the thymus and appropriate education of the developing thymocytes will occur. Tolerance may be induced by transient chimerism and subsequently

peripheral mechanisms may participate in its maintenance once the graft is in place. Organ grafts like liver, kidney and heart may be susceptible to such mechanisms since they contain passenger cells of lymphoid origin which may play a role in the maintenance of peripheral tolerance [24].

T-cells were identified as the primary effector cells for GVHD hence clinical protocols in BMT were implemented to perform TCD. However these led to increased graft failure. Facilitating cells accompanying T-cells were discovered by cell sorting technique [25]. These cells are believed to comprise about 0.4 % of whole BM population in mouse; they express CD 8 $^{+}$, CD 3 $^{+}$ and MHC class II $^{dim/intermediate}$, but are negative for α β and γ δ TCR. These are believed to facilitate engraftment without causing GVHD, since they share some markers with T-cells. They are removed during TCD process.

Gandy and Weissman reported that CD 8 $^{+}$ cell addition to pure stem cells led to decreased stem cell requirement for engraftment [26]. Complete ablative conditioning was believed to be essential until recently to achieve engraftment of allogeneic MHC –mismatched BM. However, due to its high mortality and morbidity, alternative modes for engraftment started being explored. Various strategies like monoclonal/ polyclonal antibodies for TCD, limited irradiation or immunosuppressive agents like cyclophosphamide and tacrolimus are being developed. Exner at al demonstrated that stable multilineage donor chimerism could be achieved with anti-CD 4 and anti-CD 8 pretreatment of recipient, 300 cGy total body irradiation and cyclophosphamide, 50 mg/ kg BW [27]. Engraftment was not achieved when only anti- CD4 cells were targeted, suggesting that CD 8^{+} cells in recipient are critical in rejection of allogeneic BM. In all these models, animals with 1% donor chimerism were just as tolerant as with 98 % chimerism.

AHMEDABAD EXPERIENCE

We have used nonmyeloablative conditioning in 40 renal allograft recipients (unpublished data) across MHC barriers, which include DST to stimulate the alloreactive T-cells followed by thymic transplantation of donor antigen and subsequently low dose target specific irradiation, cyclosphosphamide to delete the stimulated alloreactive T-cell clones. Polyclonal anti-T-cell antibody administration is performed subsequently to create transient ablation of donor and recipient T-cells. This is followed by BMT within 48 hours of T-cell depletion and fortified with cytokine mobilized peripheral blood stem cells.

Renal transplantation is carried out following negative LCM about 1 week after the last peripheral stem cell infusion. The total dose administered is approximately 20 x 10 ^{8} cells/ kg BW of recipient. We have observed adequate, stable allograft function with minimum/ no immunosuppression, zero rejection and no infections. They have demonstrated sustained low level (about 0.7 ± 0.5 %) mixed peripheral chimerism of myeloid and lymphoid cell lines. This level of chimerism is adequate to create donor specific tolerance without GVHD and with intact immune response to third party antigens in clinic.

CONCLUSION

There is adequate experimental work and clinical evidence which can withstand strict criteria scrutiny indicating that induction of mixed hematopoietic chimerism reliably leads to tolerance in experimental models of rodents and mice, dogs, swine, nonhuman primate model of cynomolgus monkeys and in humans. We and others have demonstrated that mixed hematopoietic chimerism can induce tolerance in human renal transplantation even across MHC barriers. We have also demonstrated that potential toxicity of conditioning regime can be dramatically reduced to zero levels without compromising its efficacy. This will definitely lead to a better and brighter tomorrow for those who are in need of organ/ cell replacement.

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Blood Transfusion and Surgery

In 1941, Agote in Buenos Aires, Hustin in Belgium and Lewisohn in the USA sumultaneously discovered that sodium citrate mixed with the donor blood was an effective anticoagulant. Subsequent major advances were the first clinical use of dicoumarol in 1941 and the introduction of warfarin in the 1950s. During the world wars it was realized that large volumes of blood could be safely stored and then transfused to an individual patient, thus paving the way for the advances in surgical techniques made in the post-war years.

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