transplantation
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IMMUNOBIOLOGY OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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ABBREVIATIONS

APC	antigen presenting cells
GVHD	graft versus host disease
MHC	major histocompatibility complex
BM	bone marrow
HSC	hematopoietic stem cells

Cells are basic elements of human body; majority of them exist and function in a terminally differentiated state. Stem cells are capable of self renewal and differentiation. They are stored in mammalian tissues and are thought to serve as a source of replacement for dying and injured cells. Adult stem cells although less plastic than the pluripotent embryonic stem cells, have the capacity to transdifferentiate in to other cell lines [1].

Hematopoietic stem cells (HSCs) are capable of inducing acquired immune tolerance in a primitive immune system [2]. Immune tolerance refers to a state of non-responsiveness to specific set of allo/ auto-antigens. Immune system of every organism has well established mechanisms primarily designed to eliminate alloantigens presented in the form of viruses, bacteria and malignant cells for self protection and survival. For the convenience of conceptual understanding mechanisms of tolerance can be divided in two major categories: central and peripheral. Central tolerance is mediated through intrathymic deletional mechanisms, basically immature T-lymphocytes generated in bone marrow (BM) will migrate to thymus on educational visa where the process of education starts when they are in the stage of double positivity having both markers, CD 4 and CD 8. These double positive cells will come in to contact with endogenous peptides bound to major histocompatibility complexes (MHC) expressed on antigen presenting cells (APCs). T-cells with high affinity receptors will interact with self-peptides and will get eliminated (deletion by negative selection process). T-cells with low and intermediate degree of affinity positively selected and will be released in to periphery to be a part of peripheral T-cell repertoire having function of immune surveillance of the organism.

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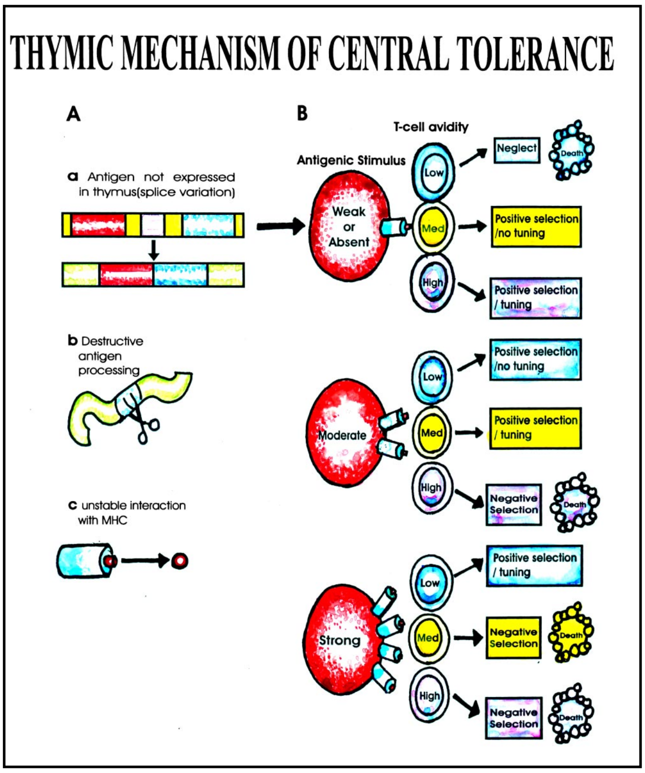

Figure-1 Thymic Mechanism of Central Tolerance.

Peripheral tolerance occurs when mature T-cells in periphery under certain conditions come in contact with antigens in the form of peptides processed by APCs and get activated to be deleted or rendered anergic. This mechanism is developed to deal with T-cells with high avidity receptors that escape thymic deletion. Thymus is expected to have a complete "all-organ DNA profile" to be shown to the T-cells while being educated.

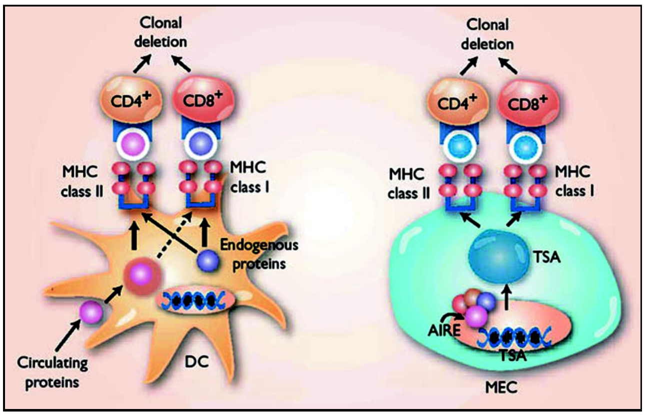

Figure-2 Know Thyself.

The educated T-cells released in periphery are anergic to all the antigens to whom the host thymus has been exposed. Central tolerance is considered to be more stable since potentially reactive T-cells are systematically deleted prior to their maturation and release in contrast to peripheral tolerance requiring actual contact of mature T-cells with the antigen. Therefore there is a concern for T-cells escaping peripheral tolerance mechanisms. It is very difficult to achieve central tolerance to alloantigens since a subset of mature T-cells in periphery have a very long life. It is imperative to delete peripheral mature T-cells reactive to alloantigens before initiating central tolerance induction [3].

One of the most reliable ways of achieving permanent robust tolerance is to have donor dendritic cells grafted permanently in thymus. The activated rejection T-cells are equipped with high avidity receptor system, and so they will be deleted in thymus by donor dendritic cells. The presence of donor T-cells in thymus will provide donor MHC antigen exposure to the developing T-cells and thus make them anergic to donor MHC antigens.

Two distinct mechanisms of tolerance have been described: deletion associated with central tolerance, and anergy associated with peripheral mechanism. Recent studies show that this division is not as distinct as was once thought. Regulatory T-cell subset phenotypically characterized as CD^{4} CD ^{25} is active in mechanisms of tolerance induction in central and peripheral pathways. These cells have their origin in thymus and have high affinity T-cell system. Somehow they escape deletion process and settle in periphery. The over-expression of the notched receptors on APCs leads to development of regulatory T-cells in response to the antigen presentation to APCs. The presence of regulatory T-cells has also been implicated in the mechanisms of "infectious tolerance".

Three patterns of chimerism emerge with different kinds of preconditioning regimes. One pattern desired by BM transplanters is to have a fully allogeneic macrochimerism where the entire hematopoietic system of the recipient is destroyed and replaced by donor HSCs leading to near complete donor hematopoietic reconstitution. Mixed hematopoietic macrochimerism is a state where both the recipient and donor hematopoietic cell population coexist in the recipient and this can be achieved with lesser intensity conditioning protocols without ablating recipient's BM. This model offers advantage over the former in the form of better immune competence and reduced susceptibility to graft versus host disease (GVHD). Of course there is a third type of chimerism described as microchimerism, which occurs in the recipients of solid organ transplantation. The transplanted organ itself serves as the source of donor cells. There is no definite correlation between presence of microchimerism and clinical tolerance. There is further information that it is not necessary to have persistence of chimerism to sustain tolerance however its presence in the initial stages is absolutely mandatory [4].

Amongst all adult stem cells, HSCs are the most extensively studied source to establish tolerance to alloantigens. HSCs are pluripotent and give rise to erythroid, myeloid and lymphoid lineages. Dendritic cells are derived from precursors of myeloid or lymphoid lineage. In rodent model it has been clearly and reliably demonstrated that establishment of mixed hematopoietic chimerism is associated with induction of donor specific tolerance evidenced by long term allograft survival without immunosuppression [5]. Tolerance is induced to specific antigens introduced to the recipient by hematopoietic precursors engrafting in hematopoietic reservoir. Presence of chimerism is not necessarily the proof of BM engraftment since some of the donor T-cells can survive in the host circulation for a very long time [6].

Clinical application of this concept for nonmalignant diseases is largely not utilized because of toxicity of host conditioning, formidable problem of GVHD and failure of engraftment. As most of the solid organ transplantations are performed across MHC barriers these problems are significantly exaggerated when utilized as an adjuvant therapy to solid organ transplantation. We believe that this situation emphasizes the need for developing nontoxic, nonmyeloablative, safe conditioning regimes specifically designed to overcome alloresistance when attempt has been made for stem cell transplantation to be grafted across MHC barriers [7, 8]. Our experience is that we need very low level of chimerism for the graft survival in renal transplantation.

Clinical transplanter will be required to design specific strategies with several steps to achieve tolerance. First of all he will be required to deal with the recipient's peripheral mature T-cell repertoire, which provides a very powerful alloresistance to the donor allogeneic stem cells. An exhaustive peripheral T-cell depletion will have to be achieved by using low dose radiation to limited lympho-hematopoietic reservoire and anti-T-cell antibodies concomitantly.

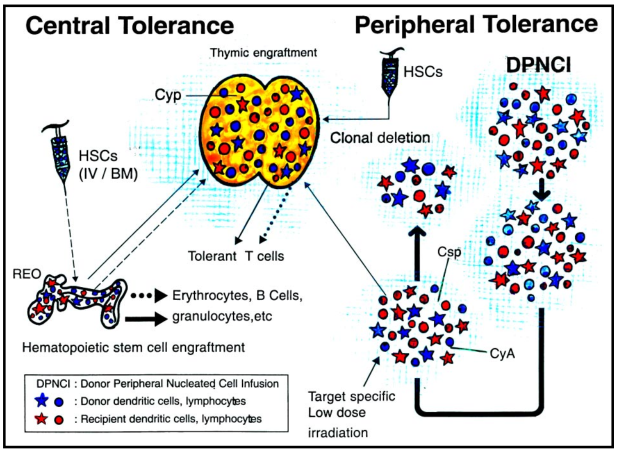

Figure-3 Concept of Tolerance

Thymic chimerism regulation is distinctly different from that of the BM, which will have to be addressed by using cyclophosphamide and cyclosporine [9, 10]. HSCs residing in the marrow (cobblestone colony forming units) are not deleted even with extreme degree of myelosuppression [3]. Busulfan, Treosulphan and radiation are the only available means to delete this subset of donor HSCs. Since the deletion is observed to be a dose related phenomenon, solid organ transplanters who aim at very low level of chimerism will be able to safely use very low dose of these drugs effectively.

The observation that mixed hematopoietic chimerism of very low magnitude is sufficient to induce tolerance in solid organ transplantation and can be achieved with safe and minimum intensity conditioning is very encouraging for people who aspire to achieve tolerance. A cocktail of above strategies will lead to success.

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