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NATURAL SUPPRESSOR CELL BIOLOGY AND ITS RELEVANCE TO "TOLERANCE" IN RENAL TRANSPLANTATION

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ABBREVIATIONS

BM : Bone marrow
NSC : Natural suppressor cells
TLI : Total lymphoid irradiation

KEY WORDS

natural suppressor cells, chimerism, renal transplantation, tolerance.

Natural suppressor cells (NSC) are the unprimed cells in bone marrow (BM), having the ability to exert non-specific suppression in various immunological responses[1, 2]. They are found at the sites of active hematopoiesis including BM, neonatal spleen and spleen of mice after total lymphoid irradiation (TLI) or Strontium administration. They are reported to prevent graft versus host disease in allogenic BM transplantation and also inhibit proliferation of murine hematological neoplasias.

Phenotypically they may have "null" surface phenotype, they may be mono nuclear cells expressing both CD34 and CD33 (CD34+, CD33+). In human marrow NSC may also produce soluble mediator(s) for suppressive activities. Culture studies have established that CD34+ CD33+ cell cultures have the most potent suppressive activity, whereas CD34+ CD33- cell cultures have little suppressive activity; and CD34- CD33- cell cultures have no suppressive activity [3]. The cells that lack lineage markers (null cells) have relatively low density (BMF γ 2:1.055-1.065 g/ml), exert greater NS activity and hematopoietic colony forming activity than the cells in other density fractions. CD 56 + cells have no effect on NS activity. NSC in mouse and monkey BM have low density and high affinity (more cell surface receptors) to wheat germ agglutinin and receptor for IL3. Hence it is anticipated that NSC are hematopoietic progenitors committed to myeloid lineage. Cells expressing CD34 molecule are thought to be multipotent and erythroid progenitors whereas CD33 expressing cells are thought to be myeloid progenitors [4].

Recently, "atypical" or "intermediate" T cells that are $CD3^{dim}$, TCRα β+, $CD4^{-}CD8^{-}$ or $CD4^{+}$ and NK1.1 which have been thought to have more "primitive" functional activity have been described in mice. It has been reported that this cell type may not be subjected to the normal (maturation/selection) process entailing migration through thymus. These cells have been

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detected in the sites of extra medullary and medullary (myeloid) hematopoiesis like liver and BM. Both immune amplifying and regulatory functions have been ascribed to these cells in context of allogenic BM engraftment [3].

Naturally occurring nonspecific suppressor cells are thought to regulate the generation of antigen-specific suppressor cells which maintain tolerance to foreign allografts. Okada and Strober have created a regulatory cell model [5]. Here they showed a two-party MLR culture containing normal responder cells and normal stimulator cells (irradiated in vivo). The responder cells generate cytolytic cells, antigen-nonspecific suppressor cells and perhaps antigen-specific suppressor cells masked by antigen-nonspecific suppressor cells. After the addition of spleen cells from adult mice given TLI or from newborn mice, the generation of cytolytic cells and nonspecific suppressor cells is blocked. However the generation of antigen-specific suppressor cells proceeds without impairment. Thus, continued alloantigen stimulation produces a pool of antigen-specific suppressor cells which continue to block the generation of cytolytic cells. The latter pool may a play an important role in maintaining tolerance in vivo even after the nonspecific suppressor cells found after TLI or in newborn mice disappear.

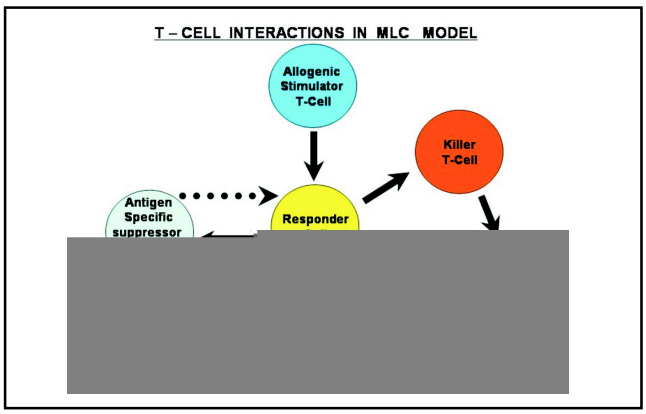

Figure 1 T - Cell Interaction in MLC Model

Figure showing a two-party MLR culture containing normal responder cells and normal stimulator cells (irradiated in vivo). The responder cells generate cytolytic cells, antigen-nonspecific suppressor cells and perhaps antigen-specific suppressor cells masked by antigen-nonspecific suppressor cells.

After the addition of spleen cells from adult mice given TLI or from newborn mice, the generation of cytolytic cells and

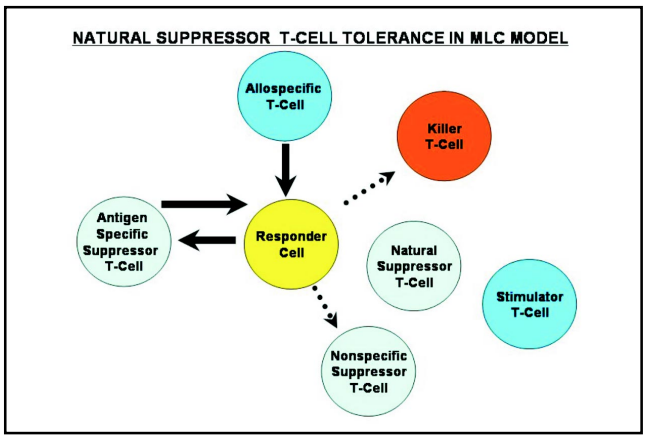

Figure 2 Natural Suppressor T - Cell Tolerance in MLC Model

nonspecific suppressor cells is blocked. However the generation of antigen-specific suppressor cells proceeds without impairment. Thus, continued alloantigen stimulation produces a pool of antigen-specific suppressor cells which continue to block the generation of cytolytic cells. The latter pool may a play an important role in maintaining tolerance in vivo even after the nonspecific suppressor cells found after TLI or in newborn mice disappear.

There is a belief that large donor BM infusions would augment natural migration of BM cell lineages into recipients and thus facilitate “microchimeric state” of BM lineages. Starzl et al believed that such chimerism is a prerequisite for tolerance, which is synonymous with a state of permanent and life long specific unresponsiveness to donor tissue alloantigens i.e. chimerism equaled tolerance, with the caveat of the intrusive presence of graft versus host disease [6].

NSC Chimerism observed in Ahmedabad Tolerance Induction protocol (table)

We evaluated NSC chimerism levels in our 100 living related renal allograft recipients who underwent tolerance induction protocol which is principally a depletion-chimerism model. We have used donor BM derived hematopoietic stem cells in unmodified form which have been fortified with peripheral blood stem cells to meet the target of $\geq 20 \times 10^8$ nucleated cells/ Kg BW (with $CD34^+$ count of 0.7 ± 0.2 %) with minimum conditioning which included low dose target specific irradiation, anti-T cell antibodies, cyclophosphamide and cyclosporin. Patients who were transplanted without tolerance induction protocol were compared with this treated group

and unstained blood samples were treated as negative controls. We studied $CD3^{dim}$ $CD4^-$ $CD\ 8^-$ $\alpha\beta$ TCR^+, $CD3^{bright}$ $CD4^-$ $CD\ 8^-$ $\alpha\beta$ TCR^+ and $CD\ 33^{weak}$ $CD34^+$ $CD45^{weak}$ cell lineages in peripheral blood at 3 monthly intervals posttransplant and in BM at 6 monthly intervals posttransplant.

We found correlation between stable NSC chimerism in $CD3^{dim}$ populations of peripheral blood, followed by BM populations and $CD33^{weak}CD34^+CD45^{weak}$ BM populations and clinical tolerance. No correlation was found with $CD3^{bright}CD4^-CD8^-\alpha\beta$ TCR^+ cell population due to fluctuation in their blood and BM levels. $CD\ 33^{weak}CD34^+CD45^{weak}$ cell population levels were very low in the peripheral blood and were statistically insignificant.

There were three kinds of operational tolerance observed. Robust tolerance where patients were living a normal life with no immunosuppression for more than 100 days was observed in 12 % patients. In these patients circulating $CD3^{dim}$ levels were stabilized at ≥ 3.6 % of total nucleated cells, BM levels were stabilized at ≥ 5.6 %. The second type of tolerance was prope tolerance achieved in 86 % patients. In these patients, $CD3^{dim}$ levels were 3.6 ± 0.2 % in periphery and 5.4 ± 0.2 % in BM. Prope tolerance is defined as early, adequate, stable graft function with no rejection episodes with minimum immunosuppression [8]. Metastable tolerance was the third kind defined as adequate graft function on minimum immunosuppression with single episode of steroid responsive acute rejection episode. It was observed in 2 % patients. In these patients, $CD3^{dim}$ levels were 2.8 ± 0.3 % in periphery and 4.6 ± 0.5 % in BM. All 12 patients with robust tolerance had unremarkable graft biopsies.

PostTx	Tn		Cn	
Percentage	$CD3^{dim}$ Periphery/ BM	$CD33^+/34^+$ Periphery/ BM	$CD3^{dim}$ Periphery/ BM	$CD33^+/34^+$ Periphery/ BM
6 months	3.6 ± 1.5/ 5.85 ± 1.5	0.09 ± 0.01/ 0.33 ± 0.1	2.05 ± 1/ 3.6 ± 1.1	0.03 ± 0.01/ 0.21 ± 0.01
1 yr	3.8 ± 1.4/ 5.6 ± 1.4	0.09 ± 0.01/ 0.33 ± 0.1	2.9 ± 0.9/ 2.5 ± 1	0.03 ± 0.01/ 0.18 ± 0.01
5 yrs	3.4 ± 1.1/ 5.9 ± 1.1	0.06 ± 0.01/ 0.71 ± 0.01	2.63 ± 1/ 1.92 ± 0.9	0.04 ± 0.01/ 0.19 ± 0.01

Table Natural suppressor cell chimerism analysis in renal allograft recipients who underwent tolerance induction protocol (Tn) vs Controls (Cn).

We have observed that significantly higher level of peripheral natural suppressor cell chimerism (>3.6 %) correlates significantly better with permanent clinical tolerance than with lower levels. This probably indicates that engraftment has occurred in the marrow. The grafted subset of cells probably originated in the donor BM and was infused in the recipient along with other cells.

To conclude, peripheral natural suppressor cell chimerism is an essential part of robust tolerance and that flow cytometry is an easy and reliable tool to measure the same. We have achieved depletion-chimerism associated tolerance in renal transplantation in Ahmedabad protocol.

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