Introduction

Renal transplantation or renal replacement therapy is the process of organ transplantation of a patient suffering from end stage renal disease (ESRD), an advance form of chronic renal failure (CRF), as the kidney is no longer able to clean wastes and extra fluid from blood. It can be deceased donor or living-donor transplantation depending on the source of the donor organ and seems to be the only treatment of choice for most patients with ESRD after hemodialysis and peritoneal dialysis. Living-donor renal transplants are further characterized as genetically related (living-related) or non-related (living-unrelated) transplants, depending on whether a biological relationship exists between the donor and recipient.

Renal transplantation was limited to human leukocyte antigen (HLA) identical siblings and was not applicable to the vast majority of patients with ESRD before the advancement of immunosuppression. The introduction of combined azathioprine-steroid therapy in 1963 produced encouraging results and became the mainstay of immunosuppression. The introduction of cyclosporine in 1983 significantly improved the outcomes of all solid-organ transplants by reducing the risk of rejection. Further innovations, including anti–T cell antibodies (both monoclonal and polyclonal preparations), as well as other maintenance immunosuppressant (eg, tacrolimus, mycophenolate, and sirolimus), have made a significant impact on both patient and graft survival. Renal transplantation is severely marked with the snag of graft failure that rates up to 15% in the first year, rising to 35% over five years and 85% in next 15 years (Hernandez et al., 2005). A complex array of simpler to severe patho-physiological phenomenon leads to the failure of renal transplant, including a cascade of immunological factors that triggers an immune response mediating rejection of transplanted organ (Poole et al., 2001); and alloantigen
independent non-immunological factors that mainly result into chronic allograft nephropathy or graft dysfunction (Suthanthiran et al., 1998). Nevertheless, the redundancy of the immune system suggests the role of several other molecular mechanisms with their resident genes playing the important effector response that contribute to the heterogeneity of the graft outcome. Currently it is quite difficult to predict therapeutic and prognostic heterogeneity of organ injury, resulting in a “One treatment fits all” approach. The confounding clinical outcomes varying from rejection to malignancy lacks the event and patient therapeutic individualization resulting into inadequate immune suppression.

1.1. Incidence of renal transplantation

The world health organization global observatory on donation and transplantation has shown the data on the incidence of ESKD that is treated by renal transplantation varies around the world (Figure 1.2 and 1.3) (Chapman et al., 2013). Recently, the chronic kidney disease registry of India (CKDRI) reported demographics for etiological spectrum, practice patterns, variations and special characteristics of CKD patients in India. About 48% of cases were in stage 5 at presentation, with the remaining in decreasing order of frequency in lower stages. Data shows that over 70% of patients require dialysis soon after presentation. However, it must be emphasized that 61% of stage 5 CKD cases were not offered any form of renal replacement therapy (RRT), 32% were on haemodialysis, 5% on peritoneal dialysis and only 2% received kidney transplantation. As haemodialysis is not widely available and deceased donor’s kidney transplantation is not well developed, live donor kidney transplantation soon after the diagnosis is the only viable and cost-effective form of long-term RRT for most patients, as the alternative for many who can’t afford to pay for dialysis is death (Kute et al., 2014).
Fig. 1.1. Number of kidney transplants per million population (Global observatory on donation and transplantation).

Fig. 1.2. Number of organ transplanted per million population (Global observatory on donation and transplantation).
The branch of organ transplantation has given several basic principles of the mechanism of acute rejection based on the analysis of selected genes, with characterization of their specific fundamental role in the immunological cascade leading to organ rejection.

However, the components of the innate immune system have been largely overlooked for a considerably long period while assessing the factors contributing to the success of an organ transplant. Of late there has been emerging evidences that the molecules expressed by the mammalian tissue grafts are recognized by the innate immune system. In this context, Natural Killer (NK) cells are emerging as an important component in the rejection process. In fact, it now appears that the innate recognition of allografts is limited to NK cells. NK cells infiltrate organ allografts (Zijlstra et al., 1992), vascular xenografts (Fryer et al., 1997), and nonvascular xenografts (Karlsson-Parra et al., 1996). They use their inhibitory receptors such as Killer Ig-like receptors (KIR) which bind to self class I MHC molecules to prevent killing of autologous cells. Mismatched allografts not expressing self MHC can therefore be potential targets for NK cell killing.

1.2. Natural Killer Cells: Component of innate immunity

Natural killer (NK) cells; an important arm of innate immunity belonging to lymphoid lineage has ability to distinguish self from non-self as well as lyses target cells and provide an early source of immunoregulatory cytokines (Robertson et al., 1990). Historic concept of NK cell suggest that, it was a large granular lymphocyte that can kill target cell “naturally,” in a spontaneous fashion without any prior sensitization and was not restricted by the target cell’s expression of major histocompatibility complex (MHC) (Herbeman et al, 1975; Kiessling et al, 1975; Lanier et al., 1986). Study in mouse models of bone marrow graft rejection (Cudkowicz et al, 1975; Murphy et al., 1987) proposed the NK cell’s ability of killing any target
lacking self-major histocompatibility complex (MHC) class I molecules (the “missing self” hypothesis) (Ljunggren et al., 1990).

Total human natural killer cells consist of approx 15% of all circulating lymphocytes. The majority of human NK cells (approx 90%) consist low-density CD56dim cells and express high levels of Fcγ receptor III (FcγRIII, CD16), whereas approx 10% of NK cells are CD56bright cells (Megan et al., 2001). Functional study by Lanier and colleagues showed that CD56dim cells are the more cytotoxic in nature during resting phase (Lanier et al., 1986). However the in vivo studies of these subsets are still needed for functional and developmental relevance.

1.2.1. NK Cells contributing to acute and chronic allograft rejection

NK cells are the well known intermediates of MHC-disparate hematopoietic stem cell rejection (Cudkowicz et al., 1971) constituting an important barrier to T cell-directed tolerance in mixed hematopoietic chimerism (Kean et al, 2006). With this notable exception of bone marrow allografts, NK cells can mediate and enhance the adaptive immune response for acute and chronic allograft rejection in several ways (Raulet et al, 2004; Kitchens et al., 2006). Firstly, in “licensing” antigen-presenting cells (dendritic cell) (DC) resulting in its maturation and subsequent T cell activation (Degli et al., 2005) with the production of IFN- that generates Th-1–like immunity by CD4 T cells (Martin et al., 2004). Secondly it augments CD4 T cell reactivity by a direct NK: CD4 T cell interaction (Zingoni et al., 2004). Together these activities contribute to graft-destructive acute T cell reactivity. In solid-organ allografts rejection, the necessity of NK cell is rare. With this notable exception, interestingly, NK cells showed its requirement to trigger rejection of cardiac allografts in CD28−/− recipients, in the absence of self MHC expression by the graft
NK cells even infiltrate syngeneic kidney transplants after ischemic reperfusion injury and may contribute to chronic graft pathology (Maier et al., 2001). Several other studies showed the contribution of NK cells to chronic allograft vasculopathy, possibly a result of the missing-self MHC class I expression by allogeneic vascular endothelium on the transplant (Russell et al., 2001). Such NK-dependent injury is IFN- dependent, a finding that correlates with the requirement of conventional CD4 T cells to mediate acute cardiac allograft rejection by and IFN-γ dependent mechanism (Wiseman et al., 2001).

### 1.2.2. NK Cells contributing to Allograft Tolerance

NK cells showed the important regulatory properties in the facilitation of allograft tolerance induction (Beilke et al., 2005). In a MHC class I-deficient, 2 microglobulin (2m) knockout mice study performed by Koller et al in 1990 and Trambley et al in 1999, NK cells showed the nature of tolerance to pancreatic islet allografts after host treatment targeting either CD154 or CD11a (LFA-1) as CD8 T cells have been shown to constitute a barrier to allograft tolerance induction after co-stimulation blockade concluding the role of NK cells, inspite of NKT cells in the allograft tolerance induction. The concluding remarks provide an insight into the NK cell regulatory elements and their effect on the outcome of renal transplantation. It is found that the activity of NK cell is regulated by its cell surface receptors (activating and inhibitory) known as Killer Immunoglobulin like- receptors that recognizes the MHC class I and class I-like molecules expressed by the target cells. These receptors largely recognize HLA-A, B and C, ubiquitously present on all nucleated cells, in an allele-specific manner and transduce an array of activating and inhibitory signals to the NK cells. The activity of NK cell is governed by the balance between these two receptors (in presence of a net activating
signal, NK cell mediated cytotoxic killing of the target cell will occur and vice versa). The genes for these KIRs are on chromosome 19 and therefore inherited independently of HLA (chromosome 6).

1.3. Regulation of NK cell activity by Killer Immunoglobulin-like Receptors:

The killer immunoglobulin-like receptor found on surface of natural killer cells, some CD8 + T cells and CD4 + CD28 null T-cells (Moretta et al., 2004). The family is comprised of 17 genes, 15 of which are expressed and two are pseudogenes, arranged in a head to tail fashion over a 150 kb region on 19q13.4. All 17 genes are believed to have evolved from a single ancestral KIR gene after the divergence of the hominoid line from mice (Rajalingam et al., 2004), as mice have only two KIR like genes and instead rely on the Ly49 family of C-type lectin receptors for NK cell regulation.

The KIR haplotypes have been repeatedly shuffled by reciprocal and non-reciprocal crossing over events and thus, aside from the four ‘framework’ genes, which are present in all individuals (KIR3DL3, KIR3DP1, KIR2DL4 and KIR3DL2), they are highly variable with respect to the number and types of genes they contain (Uhrberg et al., 2005).

1.4. Definition of the problem

In the present study we will analyze 277 ESRD patients who underwent kidney transplantation and their donors; further 75 donor-patient pairs who suffered a rejection episode after transplantation will be studied. Both the groups will be analyzed for their KIR genotypes and the profiling so obtained will be correlated with the HLA status of the pair and outcome of the transplant. The particularly susceptible as well as protective combinations obtained will further be dissected for the allelic variants of
KIR genes through direct sequence-based typing. Overall, the study will focus on the following important aspects:

1. Analysis of the KIR profiling in both the groups is intended in order to gain a deeper understanding of NK cell regulation and its effect on the outcome of the organ transplantation.

2. Natural killer cells have recently been unexpectedly implicated in the tolerance of solid organ allograft. This finding has led us to believe that the extent of closeness at the level of KIR together with that of HLA would influence the balance of NK cell activity in the recipient in a manner that could contribute to the tolerance of the allograft.

3. High resolution allelotyping of HLA class-I genes and selected KIR genes among the donor-patient pairs of both the groups would allow us to comprehensively compare and analyze the potentially susceptible as well as protective combinations of KIR and HLA taken together. This is expected to explain to an extent, the different outcomes observed in different patients in our experience. Identification of risk factors that influence the incidence and severity of acute rejection remains the priority of transplant biologists and this study can contribute an additional tool of donor-recipient matching prior to going for transplantation.

1.4.1. Objectives of the study

1. To analyze the KIR gene content in donor-patient pairs in both the groups by gene specific PCR amplification (PCR-SSP)

2. High resolution genotyping of HLA class-I antigens (HLA-A, -B and -C) in donor-patient pairs in both the groups by allele specific PCR amplification (PCR-SSP) kits
3. Analysis of KIR-HLA compound genotype among both the groups with emphasis on the clinical correlation with the graft outcome after transplantation