Discussion

In solid organ transplantation, NK cells show the ability to distinguish allogeneic MHC antigens in conjunction with KIR receptors. NK cells recognize HLA present on all cells through activator and inhibitor cell surface receptors, including KIRs. All KIRs are randomly expressed on NK cells, which are educated to self-HLA. This event occurs when the mature KIRs vary genetically among individuals and the balance of KIR activating and inhibiting signals regulate NK cell function. Donor NK cells mediate an anti-allograft effect in recipients when inhibitory KIRs are mismatched for HLA type I transplants since these cells recognize recipient allograft cells as foreign (Agrawal et al., 2008). The mismatched donor NK cells can decrease the rate of relapse. However, the mismatched transplant also has an increased risk of graft versus host disease (GvHD). NK cell function is regulated by KIR interactions with matched HLA class I alleles. For inhibitor KIRs, binding with matching HLA prevents donor NK cell activation to self. For activating KIRs, donor NK cells that bind the matched HLA are activated and induce cell lysis in the recipient transplant. The same approach has been applied to test whether HLA epitope disparity influences NK alloreactivity of the donor in the renal transplant setting. (Tran et al., 2005)

Killer cell immunoglobulin-like receptors (KIRs) are the cell surface receptors found on natural killer cells and in a small number on T-lymphocytes. These KIRs exhibit two (KIR2D) to three (KIR3D) immunoglobulin-like extra cellular domains with short or tall cytoplasmic tail having immunotyrosine based inhibitory (ITIM) or activating (ITAM) motifs. Those KIRs having long cytoplasmic tails containing ITIM motifs provides inhibitory signals whereas that having short cytoplasmic tail lacks ITIM, hence associate with adaptor molecules containing ITAM motifs and
producing activation signals upon binding with their appropriate HLA class I ligand. KIR encodes up to 14 genes present in different combinations among different individuals, associating several human diseases. Normal cells of the body possessing HLA class-I molecules that binds with their inhibitory KIR counterparts escapes the act of natural killer cell killing. Whereas the transformed or infected cells showed the lesser or diminish amount of HLA class I on their surface, are prone to NK cell attack. Some of the activating KIRs also bind HLA class I molecules, albeit with lower affinity, but their physiological ligands are still known (Parham et al., 2012). To some extent KIRs display ligand specificity as compared to T-cell receptor. There are various KIRs with two extracellular immunoglobulin like domains that binds with HLA-C molecules, depending on their amino acid residue at 80th position. Among these, KIR2DL2 and KIR2DL3 bind HLA-C1 molecule with asparagine at 80th position whereas KIR2DL1 and KIR2DS1 binds HLA-C2 allomorphs with a lysine at 80th position. Recently Parham et al., showed that KIR2DL2 may also bind some C2 molecules in addition to C1 (Parham et al., 2012). With some exception already noticed in KIR association, its involvement in of renal allograft has not been extensively studied. Their results are not concordant: some authors found no effect of KIR genotype, whereas others detected protective effect of KIR2DL2/KIR2DS2 or KIR–KIR ligand mismatch. We have found the HLA-Bw4/3DL1 matched cases prominently among ESRD cases (~53%) showing a ~11 years of survival followed by HLA-C2/2DL2 matched cases (~41%) showing a survival of ~7 years. Interestingly, in patients, whose end stage renal disease was caused by glomerulonephritis, the effect of KIR2DS4 was stronger than HLA mismatch, whereas opposite was true for recipients with other causes of renal failure. It has been reported that NK cells would recognize donor cells
as self, and would develop a HLA environment which is similar to that of the donor. However, in poorly matched donor-recipient pairs, recipients NK cells may not necessarily recognize donor cells as self. We have found the HLA-Bw4/3DL1 matched cases prominently among ESRD cases (~53%) showing a ~11 years of survival followed by HLA-C2/2DL2 matched cases (~41%) showing a survival of ~7 years. While the activating KIR receptor associated HLA-Bw4/3DS1 and HLA-C1/2DS1 combinations showed a reduced survival rate among the rejection cases. The combinatorial analysis revealed protective association for HLA-Bw4/3DL1.

Acute renal allograft rejection of transplanted kidney is mediated by alloreactive T cells that recognizes both HLA alloantigens (most importantly, HLA-A, -B and -DR) and minor histocompatibility antigens (Nankivell and Alexander 2010; Sun et al., 2011; Womer and Kaplan 2009). However, NK cells are also found to infiltrate renal allografts (Sun et al., 2011; Totterman et al., 1989). An ex vivo study showed the increase of cytotoxicity in renal allograft recipient’s NK cells against donor cells three days after transplantation. This study suggests that cytotoxicity depends on activating KIR genes in the recipient, potentially recognizing the HLA of donor (Vampa et al., 2003). Moreover, the rejected allograft of recipient’s peripheral blood contained higher numbers of NK cells than blood of nonrejectors (Cooksey et al., 1984). In this regard, several groups studied possible combinations of KIR and KIR ligand genes associated with renal allograft rejection. An extensive study on 2,757 donors and recipients from different continents did not detect any effect of compatibility and incompatibility of KIR and HLA (Tran et al., 2005). Kreijveld et al., (2007) also found a similar negative result when typed for KIR ligand and KIR genes both. However, among several studies, two of
them revealed protective association of recipient KIR2DL2/KIR2DS2-positive genotype, particularly when donor was having a combination of KIR2DL2-HLA-C1 (Cirocco et al., 2007; Kunert et al., 2007). On the other hand, study on English patients showed better and longer graft survival when the recipient have association with HLA-C2 (Hanvesakul et al., 2011). Interestingly, the effect of KIR–KIR ligand mismatch in HLA-identical recipient–donor pairs was as strong as that of HLA-A, -B mismatch in pairs matched for HLA-DR only (van Bergen et al., 2011).

In our study the rejection cases were classified on the basis of serum creatinine level into true rejection (S.Creatinine > 6 ml/min/1.73m^2) and rejection with a functioning graft (S.Creatinine< 6 ml/min/1.73m^2) and subsequently compared with the KIR-HLA match-mismatch criteria upon which we have found individuals where rejection with functioning graft showed higher survival rate as compared to the individuals under the true rejection category. To conduct this study we have taken 277 north Indian renal transplant recipient and their respective donors for analysing individual KIR gene frequencies. The Individual gene carriage frequency among 277 renal transplant patients with their donors, showed significant protective association for KIR2DL1 gene (p= 0.0317, OR= 0.72, 95%CI= 0.57-0.90) whereas on comparing patients who underwent rejection (both chronic and acute) with those of non-rejection cases we got almost two fold risk association with KIR2DS4 gene (p= 0.0413, OR= 1.91, 95%CI= 1.02-3.57).

Ruggeri et al., first illustrated the KIR ligand incompatibility or HLA epitope mismatch is due to the absence of donor HLA-I alleles in the recipient which leads to NK alloreactivity in the graft versus host (GVH) direction resulting in protection against graft rejection, relapse, and graft versus host disease (GvHD)( Agrawal et al., 2008). The same approach has
been applied to test whether HLA epitope disparity influences NK alloreactivity of the donor in the kidney transplant setting (Tran et al., 2005). Earlier reports also suggested that donor cells having one homozygous HLA-Cw polymorphism would be at risk for lysis by recipient NK cells if the recipient is either heterozygous or homozygous. For the other HLA-Cw polymorphism patients mismatched for HLA-Cw may not have the correct repertoire of KIR to enable them to be activated by donor cells. Moreover, recipients heterozygous for HLA-Cw would recognize donor cells from either group as self and therefore should not be considered allospecific. Certain combinations of KIR-HLA haplotypes have also been linked with susceptibility to the risk of preeclampsia (Faridi et al., 2009), HIV infection (Martin et al., 2002), or autoimmune diseases (Sashtri et al., 2008; Yen et al., 2001), but susceptibility could be also due to reduced NK cell inhibition, when an individual is homozygous for HLA-C1 alleles and lacks a ligand for KIR2DL1 and those homozygous for HLA-C2 lack the corresponding ligand for KIR2DL2/3.

In the present study inhibitory receptor KIR2DL1 showed protective effect for ESRD while susceptibility was concurred against KIR2DS4 for rejection cases. The combinatorial analysis revealed significant protective associations against KIR2DL2-HLAC1, KIR2DL3-HLA-C1 and KIR3DL1-HLA-Bw4 combinations for ESRD patients.

KIR, the best characterized group of NK receptors are allotype and isotype specific. Thus, allografts with mismatched HLA molecules can potentially be recognized and killed. KIR and HLA are present on different chromosomes and therefore are differently inherited. It has been earlier reported that allogeneic cells will be subjected to NK-cell killing if their class I MHC molecules are not recognized by the recipient’s inhibitory
receptors. NK-cell activity is probably a result of the balance between activating and inhibitory receptors (Lanier et al., 2001).

Much less is known about the comparative expression of inhibitory and activating KIRs on T-cell subsets, and there are currently no data on T-cell expression of KIRs in the renal post-transplant period. However, a current study (Prakash et al., 2013) provided evidence that inhibitory KIRs change the T-cell effector profile. Although granule release and cytotoxicity remain unaffected, the transcription of T-cell receptor (TCR) relevant genes (e.g., IFN) gets affected. KIR expression may enable T cells to use cytotoxic mechanisms without causing collateral damage through the production of cytokines and without distorting T-cell homeostasis through clonal expansion. There exists a potential clinical role of certain KIR/HLA ligand interactions in renal transplantation whose biological basis remains uncertain as the genetic study cannot consider functional aspects of KIR receptors in the clinical setting of renal transplantation. The imbalance between stimulatory and inhibitory signals might support graft rejection.

Furthermore high resolution molecular typing of HLA-Bw4 specific KIR3DL1/3DS1 gene among 100 renal transplant patients and their respective donors were performed by direct sequence-based typing (Applied Biosystems) of exons 3, 4, 5, 6, 7 and a flanking region containing exon 8 and 9 of the KIR3DL1/DS1 gene complex. The allele 3DL1*0010101 was found to be the most commonly occurring allele, followed by 3DL1*00402, *008, *00401 and *01501. 3DL1*0010101, showed protective association (OR=0.37, p-value=0.0072, 95% CI=0.18-0.74) among renal transplant patients and donor as well as among rejectors and non rejectors group (OR=0.17, p-value=0.0170, 95% CI=0.02-1.16). The occurrence of homozygosity for these alleles was a rare event.
The ten KIR3DL1/DS1 alleles found in the present study among rejectors and non rejectors groups have been analyzed with their HLA-Bw4 ligand association. No Significant associations were found for both the group with KIR-HLA combination. Further by classifying the study population on the basis of HLA-B sub allele classes HLA-Bw4 and HLA-Bw6, none of the combination showed significant association.

Still further studies have to address the receptor ligand interaction in the context of solid organ transplantation in more detail to understand how HLA-KIR genotypes contribute to transplant outcome. Therefore, to analyze graft infiltrating NK cells or T cells activity towards KIR expression and to study their interaction pattern with ligand-expressing cells may ease the prognosis of renal allograft rejection and graft survival.

**CONCLUSION:**

These study findings suggest that a particular set of activating KIR receptor-HLA ligand repertoires might predispose to acute allograft rejection, while another set of inhibitory KIR receptor- HLA ligand repertoires might play a role in prolonged allograft survival. Studies are required to address the receptor ligand interaction in the context of solid organ transplantation vividly in order to understand how HLA-KIR genotypes contribute to transplant outcome, which may ease the graft survival and prognosis of renal allograft rejection.