Killer Immunoglobulin-like Receptor (KIR) genotype and the risk of viral infection after kidney transplantation

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Abstract

Introduction: Natural killer cells (NK) represent the first line of defense against infections and tumors by direct cytotoxicity but also modulating an existing immune response by secretion of soluble factors such as cytokines. The functions of NK cells are regulated through a complex repertoire of molecules expressed on their surface, receptors specific for HLA class I molecules.

Material and methods: The study was performed on 80 kidney transplanted patients in 2009. Before transplantation, viral screening in both, donors and receptors, was performed using serological tests and posttransplant, quantitation of CMV-DNA in patients was done by molecular biology technique (Real-Time PCR). PCR methods were used, also, for HLA and KIR genotyping.

Results: In the first three months posttransplant, the incidence of CMV infection in our study was 36.3% (29 patients). Viral load values were between 76 and 200,000 genome copies/ml but most of them were below 10,000 genome copies/ml. Among patients with "missing KIR ligands" only two (9.5%) was detected with CMV infection compared with the others patients where the percentage was much higher (45.8%).

Conclusion: Analysis of the KIR and HLA genotyping in these patients suggested that absence of HLA ligands for KIR inhibitory receptors and a high number of KIR activating receptors could be associated with a significant reduction of CMV infection.

Key words: kidney transplant, NK cell, KIR genes, CMV infection

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Introduction

Natural killer cells (NK) components of innate immune system represent the first line of defense against infections and tumors by direct cytotoxicity but also modulating an existing immune response by secretion of soluble factors such as cytokines. The functions of NK cells are regulated through a complex repertoire of molecules expressed on their surface, receptors specific for HLA class I molecules. Until now were identified three distinct family of genes encoding for synthesis of these receptors: (1) ***KIR receptors*** (killer cell Ig-like receptors) (2) ***ILT receptors*** (immunoglobulin like transcripts), recently discovered, are expressed mainly on the surface of B and T lymphocytes and myeloid cells, but several members of this group are found on NK cells. In literature, they are also called ***LIRs*** (leukocyte Ig-like receptor) and ***MIRs*** (macrophage Ig-like receptor) receptors (3) ***C- type lectins***.

First two types of receptors are members of the immunoglobulin superfamily (Ig) and have a similar structure, while lectins are heterodimers resulting by covalent binding of a CD94 molecule with a molecule from NKG2 receptors family.

Each of these three families of receptors have several distinct members that have identical or similar ligands, receptor-ligand interaction causing activation or inhibition of NK cells [1,2]. Many data show that NK cells and their receptors have an important role in the development of many diseases (especially autoimmune diseases), in transplantation, pregnancy or in viral infections control [5-7].

Our study focuses mainly on KIR genotyping in donors and kidney transplant recipients and its impact on posttransplant outcome, in terms of rejection phenomena and viral infections development.

KIR receptors

Are members of the immunoglobulin superfamily and encoding genes are located on long arm of chromosome 19q13.4, in the Leukocyte Receptor Cluster (LRC). So far were identified 14 expressed genes and two pseudogenes. *Generally, the receptor structure consists of two or three extracellular domains (2D or 3D), a transmembrane portion and an intracytoplasmic tail which can be short (S) or long (L).* The nomenclature used is based on this structure (*e.g.*: KIR2DL1 or KIR2DS4).

Activation of KIR receptors with intracellular long tail exercises an inhibitory effect on NK cell functions (except 2DL4 that has a stimulatory action), while KIR receptors with short intracellular domain transmit activating signals through another molecule, DAP-12 (Fig.1).

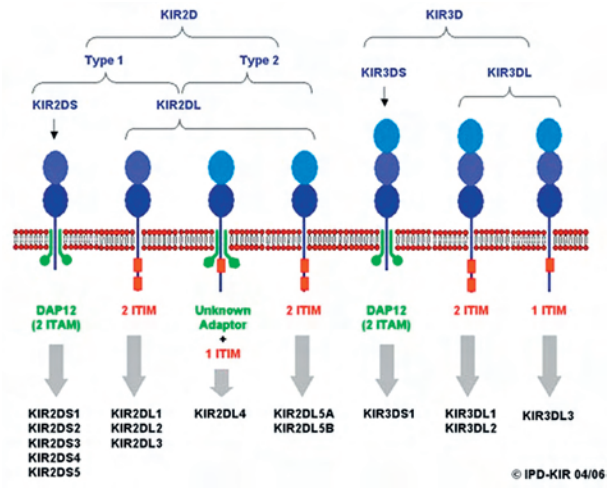


Fig.1. KIR structure and nomenclature (A. P. Williams - KIR and Their Role in Disease, Southampton, UK, 2006)

On a chromosome genes are organized in a head-to-tail fashion and in this context *are described* two possible haplotypes (A and B). Haplotype A has seven loci: 2DL1, 2DL3, 2DL4, 3DL1, 3DL2, 3DL3, 2DS4. The most functionally relevant distinction between haplotypes A and B is the number of stimulatory receptors present. Haplotype A *contains only a single stimulatory KIR gene (2DS4), whereas haplotype B contains various combinations of 2DS1, 2DS2, 2DS3, 2DS5, 3DS1 and 2DS4 (Fig.2).* Furthermore, the 2DS4 gene has a null allele with a population frequency of about 84%. Thus, some individuals are homozygous for an A haplotype from which no activating KIR is expressed [2-4]. The frequencies of haplotypes A and B are roughly equal in Caucasian population, but on the basis of gene content, haplotype B displays a much greater variety of subtypes.

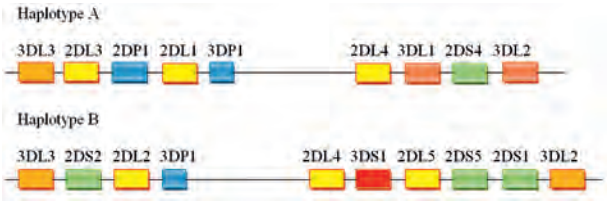


Fig.2. Gene order of two fully sequenced KIR haplotypes (M. Carrington, P. Norman - The KIR gene cluster, 2003)

As shown in the figure, regardless of haplotype, KIR genes are organized in two regions, around three conserved framework genes: 3DL3 and 3DL2 at either end and 2DL4 in the middle. *In these regions, the genes content may vary and duplication, gene recombinations*

or deletions are likely facilitated by the close proximity of the genes (about 2 kb) and the sequence similarity of the intergenic sequences. Each gene may have several alleles, between four (KIR 2DS1) and nineteen (KIR 3DL1) [3,4].

Regarding KIR ligands, they are well defined in some cases (Fig.3).

- Kir 2DL1 binds to a subset of HLA-C molecules having the aminoacid Lysine at position 80 of heavy chain (HLA-CLys80 or HLA-C2).
- KIR 2DL2 and KIR 2DL3 recognize other HLA-C allotypes with Asparagine at position 80 (HLA-CAsn80 or HLA-C1).
- KIR 3DL1 ligands are HLA-Bw4 allotypes, representing almost one third of the HLA-B alleles.
- KIR 3DL2 binds to HLA-A3 and HLA-A11.
- KIR 2DL4 probably interacts with HLA-G molecules but the physiological role of this
- interaction remains unclear.

The specificities of activating KIR receptors are not yet well defined because of much lower affinity to HLA class I antigens. Based on structural similarity, is assumed that KIR 2DS2 and 2DS1 had the same ligands as KIR 2DL1, respectively KIR 2DL2. In other receptors, the specific ligands have not yet been identified [5,6].

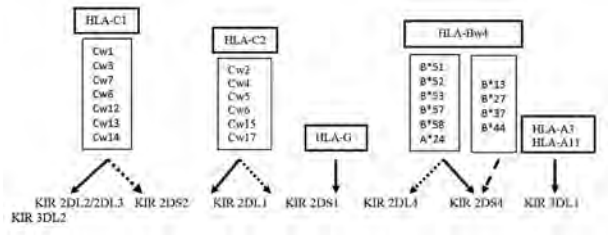


Fig.3. HLA KIR ligands

KIR diversity is also compounded at a functional level by the diversity in the HLA ligands for KIR. The MHC locus in which the HLA class I ligands for KIR reside is on chromosome 6 and, thus, the KIR and its ligands segregate independently at meiosis. As KIR recognize only subset of HLA class I molecules, a given individual may have a specific KIR, but may lack the corresponding HLA class I ligand; therefore, in a given individual, a specific KIR may be nonfunctional [8].

The balance of activating and inhibitory signals is crucial in regulating natural-killer cell effector function and the absence of HLA class I molecules expression can induce susceptibility of a potential target cell to NK-mediated cytolysis [9,10].

Human genetic studies have shown that KIR / HLA genotype with an activating profile (i.e. the presence of activating KIR receptors or a lack of inhibitory KIRs or its specific ligands) is associated with a delayed progression of AIDS [11] and increased incidence of recovery from hepatitis C infection [12]. In a number of studies, including rodent models of CMV infection [13,14] and after allogenic stem cell transplantation [11], have been highlighted the pivotal roles of NK cells and KIR receptors to eliminate CMV virus.

Cytomegalovirus infection is the most common viral complication following allogenic hematopoietic stem cell transplantation (HSCT) and solid organ transplantation (SOT) (liver, kidney). SOT is of special interest because current immunosuppressive protocols target the activation of T cells and therefore impair specific antiviral immunity. In contrast, NK cell function appears to be unaffected by such therapeutic regimens and could therefore potentially play an important role in the anti-CMV immune response [17-19].

Material and methods

Patients

The study was performed on 80 kidney transplanted patients in 2009, 28 women (35%) and 52 men (65%) with median age of 41 years (16-59 years). Patients with previously failed graft and steroid-resistant acute rejection episode, anti-HLA sensitized patients and patients with bone marrow depression were excluded.

CMV monitoring

Before transplantation, the presence of anti-CMV IgG antibodies was determined by microparticles enzymatic immunoassays - MEIA (AxSYM, Abbott Diagnostics) in both, patients and donors.

For identification and quantitation of CMV-DNA in samples of DNA extracted from plasma or whole blood collected in EDTA, we used a quantitative assay based on molecular biology technique, Real-Time PCR (Cepheid, Nanogen Advanced Diagnostics). An amplification reaction specific for a region of the CMV MIEA gene (major immediate early antigen) is performed and then a FAM fluorophore-labelled probe specific for CMV becomes activated once it hybridises with the specific product of CMV amplification. The fluorescent emission increases as the amplification reaction specific product increases and is measured and registered by the instrument. The analytical

sensitivity of assay allows the quantification of approximately 1000000 to approximately 20 molecules of target DNA in the 5µl of extracted DNA.

HLA and KIR genotyping

DNA was obtained from whole blood collected on EDTA using QIAmp DNA Mini Kit. HLA-B and –C was performed by molecular techniques, SSOP (sequence specific oligonucleotides probes – Innogenetics kits) and SSP (sequence specific primers – Invitrogen kits). SSOP technique involves a locus specific amplification followed by hybridization with specific probes for HLA alleles identification. In *SSP technique, amplification is done with specific primers for a particular HLA allele and PCR products are identified using agarose gel electrophoresis.*

KIR genes were analyzed by PCR-SSP using the KIR Genotyping SSP Kit (PEL-FREEZ, Dynal Biotech) which included the inhibitory receptors 2DL1, 2DL2, 2DL3, 2DL5, 3DL1, 3DL2, 3DL3, the activating receptors 2DL4, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DS1 and pseudogenes 2DP1 and 3DP1 (not expressed).

Results

After serological CMV (IgG) screening, in both, donor (D) and receptor (R) the following combinations were obtained: R+/D+ 37 cases (46.3%), *R+/D- 19 cases (23.3%), R-/D- 10 cases (12.5%) and R-/D+ 14 cases (17.5%).*

KIR receptors typing in transplanted patients have shown that 23 individuals had haplotype AA (28.75%) and the others were haplotype AB or BB (71.25%). These percentages overlap those reported from other population studies [20].

Patients with missing KIR ligands were defined as individuals lacking either HLA-C1 or HLA-C2 and/or Bw4 ligands for which the corresponding inhibitory KIR is present. *In our study almost one third of patients (21 recipients - 26.3%) fell under this category.* Patients with no missing LIR ligands are heterozygous HLA-C1/HLA-C2and HLA-Bw4 – positive.

In the absence of any preventative therapy, CMV infection occurs in approximately 30-75% of transplant recipients, with an incidence of CMV disease of between 8 and 80%, depending on the type of transplantation, immunosuppression and, most importantly, the donor/recipient (D/R) CMV serostatus [21,22]. In the first three months posttransplant, the incidence of CMV infection in our study was 36.3% (29 patients). *Note that, previously transplantation, nine of these patients had seronegative with seropositive donor and one seronegative patient had seronegative donor.* Viral load values were between 76 and 200.000 genome copies/ml but most of them were below 10.000 genome copies/ml (Fig.4).

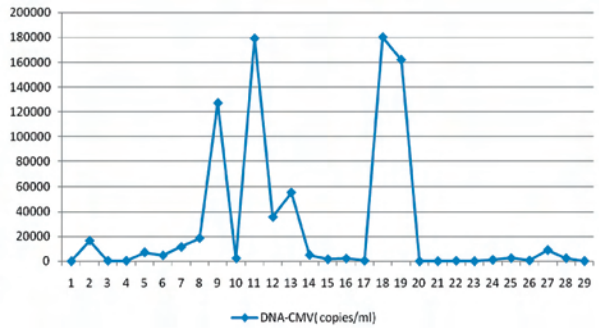


Fig.4. Viral load at 3 months posttransplant

This relatively low incidence of CMV infection can be explained by the fact that, in accordance with international protocols, before transplantation patients received prophylactic antiviral therapy. *However, in patient who was seronegative with seronegative donor but presented posttransplant CMV infection, with low viral load (102 genome copies / ml) that infection probably occurred, due to immunosuppressed, after the environment exposure or the donor had anti-CMV IgG very low titers that could not be detected.*

Among patients with "missing KIR ligands" only two (9.5%) was detected with CMV infection compared with the others patients where the percentage was much higher (45.8%) (Fig.5). Since inhibitory KIR receptors have no specific ligands, they became inactive, nonfunctional. Therefore, predominantly remains active activating KIR receptors and NK cell received activation signals leading to increase antiviral immunity by unspecific cytotoxicity mediated by natural-killer cells.

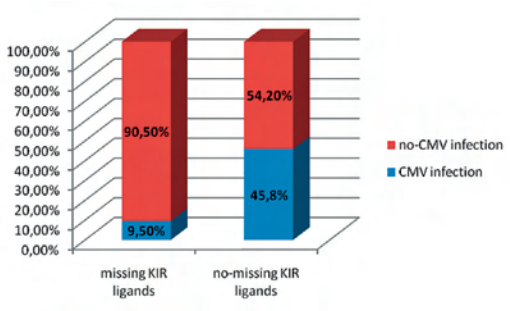


Fig.5 Missing KIR ligands are associated with a lower incidence of CMV infection after kidney transplantation.

Discussion

In this study, we investigated the link between NK cells and CMV infection in a cohort of 80 kidney-transplanted recipients. Analysis of the KIR and HLA genotyping in these patients suggested that absence of HLA ligands for KIR inhibitory receptors and a high number of KIR activating receptors could be associated with a significant reduction of CMV infection. During the first months after transplantation the rate of CMV infection is higher because the immunosuppression is stronger and the protective effect provided by the missing KIR ligand is predominant during the first three months but decrease over time.

The role of NK cells in transplantation has been highlighted in a number of studies on allogenic hematopoietic stem cell transplantation (HSCT), where the absence of HLA ligands for inhibitory KIR (missing KIR ligand) or a large number of activating KIRs has been associated with a lower rate of relapse, GVHD and CMV infection [9,15,16]. In SOT, recent studies suggest that specific KIR/HLA class I ligand combinations between donor and recipient might influence short-term graft outcome after renal transplantation [23,24].

However, KIR/HLA ligand genotypes have proven quite complex, and human genetic studies confirmed by recent *in vitro* human data support evidence that distinct KIR/HLA compounds could be associated with different susceptibility to infectious disease or to tumor cells. Human NK cells that have matured in the presence of HLA ligands for KIR receptors developed more potent cytotoxic capacity than those maturing in the absence of such ligands through a mechanism known as "licensing" [25]. The contribution of non-KIR family receptors could also become predominant in the immune response of NK cells to viral infections *in vivo*.

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