Pas de deux: Natural Killer Receptors and MHC Class I Ligands in Primates

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Abstract: Major histocompatibility complex (MHC) class I and NK cell receptor gene regions are a paradigm of genomic plasticity as they reveal a considerable degree of diversity, exemplified by high allelic polymorphism, genomic duplications and contractions, and formation of gene families. Both genetic components show signs of rapid evolution due to strong selective pressure to combat pathogens. Comparative analyses of these genomic regions in various primates revealed considerable differences, reflecting species-specific adaptations to pathogenic threat or different strategies to combat infections. MHC and NK receptor genomic diversity in populations are important factors that determine susceptibility or resistance to a variety of diseases including autoimmune and infectious diseases as well as reproductive success.

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The Major Histocompatibility Complex (MHC)

The major histocompatibility complex (MHC) was initially identified 70 years ago by Peter Gorer during studies of blood group antigens and of tumour transplantations in the mouse [1,2]. The hallmark of the MHC is its extraordinary polymorphism, resulting in considerable differences between unrelated individuals and, usually, in rejection of transplanted organs and tissues. Besides its striking role in transplantation, the MHC could be shown to contain genes that essentially determine immune responsiveness, previously called IR genes. The MHC is particularly characterised by the class I and class II genes (= the IR genes), which are multiplicated and are the most polymorphic genes in the mammalian genome. For example 830 alleles of the *HLA-B* gene are currently stored in the IMGT/HLA database (see <http://www.ebi.ac.uk/imgt/hla/stats.html>). The clustering of class I and class II genes has been used to divide the MHC into regions [3]. In the human MHC, which maps to chromosome 6p21.3 [4] and is also designated human leukocyte antigen (HLA) complex, the class I and class II regions are localized at the telomeric and centromeric end of the MHC, respectively [5,6]. The MHC turned out not only to contain the most polymorphic genes, but also to be the gene-densest region of the human genome, with more than 220 genes identified in 3.6 Mb [7].

Class II molecules consist of MHC-encoded α and β chains [8] and are expressed at the cell surface of professional antigen-presenting cells such as dendritic cells, monocytes, macrophages, B cells and thymic epithelium. Their function is to bind peptides of about 13-17 amino acids, which are derived from exogenous antigens, and to present them to CD4⁺ T helper cells [9]. Most MHC class II genes are polymorphic, the most polymorphic human class II gene

being *HLA-DRB1* with currently 463 known alleles in the human population (see <http://www.ebi.ac.uk/imgt/hla/stats.html>). Haplotypic differences in class II gene content are known for many vertebrate species including primates [5,10], further contributing to genetic diversity at the MHC.

Class I molecules are expressed on the surface of nearly every cell of the body and consist of an MHC-encoded α chain and the non-covalently bound beta-2-microglobulin, which is not encoded in the MHC. Class I molecules bind peptides of 8-9 amino acids usually of endogenous origin such as self- or virus-derived peptides, which are presented to CD8⁺ cytotoxic T cells. Besides this classical antigen presentation function, MHC class I molecules serve as ligands for receptors of natural killer (NK) cells [11-13], thus being essentially involved in adaptive and innate immunity. MHC class I molecules have further immunological and also non-immunological functions [14-18], which will not be reviewed here.

The MHC class I genes in mammals are organised in clusters of genes that map between non-MHC class I genes (Fig. 1), which are also referred to as framework genes or anchor genes [19,20]. Although class I genes of species belonging to different genera are usually paralogous [21], various class I gene clusters map exactly to the same position in different species with respect to flanking framework genes [19,20,22,23]. MHC class I genes exhibit a high degree of turnover. Frequent duplications and deletions due to non-reciprocal recombinations shape the class I gene contents of most species [24] and even haplotypes of various species such as rhesus macaques [25,26], mice [27] and rats [28,29]. Yet, many of the amplified class I genes are only weakly expressed and are classified as non-classical class I (or class Ib) genes. In addition to their restricted expression pattern, class Ib genes are usually not polymorphic. In contrast, classical class I (or class Ia) genes are ubiquitously and strongly expressed and are polymorphic. Thus, even though the MHC of some species can exhibit many class I genes (Fig. 1), only

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Fig. (1). Schematic genomic organisation of the *HLA* region on human chromosome 6p21.3 and comparison to the *BAT1-POU5F1* interval in the rhesus macaque [25]. The rhesus macaque *MHC* maps to chromosome 6q24 [93]. Members of the MHC class I gene family are indicated by hatched boxes. Only the symbols of functional genes are denoted.

few (usually 1 to 3) are found to represent class Ia genes, the products of which perform the classical immunological functions of antigen presentation and as ligands of NK cell receptors. With few exceptions, e. g. *HLA-E* or *MICA*, the role of the many class Ib genes still remains enigmatic.

Two members of the class I gene family, which are distantly related to class Ia and to MHC-linked class Ib genes, are tightly linked to *HLA-B* [30,31]. These are the MHC class I chain related *MICA* and *MICB* genes that encode stress-inducible cell-surface molecules, which do neither bind beta-2-microglobulin nor present any peptides [32]. Expression of MIC molecules is usually restricted and can be found for example on epithelial cells and frequently on tumour cells. Interestingly, expression of MIC genes can be upregulated upon certain stimuli ('cell stress') such as viral infections [33], oxidative stress [34], or heat shock [35]. Furthermore, expression is activated by the NF-kappa B pathway [36] and by DNA damage [37]. *MICA* and *MICB* molecules could be shown to be ligands for the strong activating receptor NKG2D (KLRK1) [38,39], which is expressed on NK cells, $\alpha\beta$ and $\gamma\delta$ T lymphocytes, and macrophages. Further distantly related members of the MHC class I family are the UL16 binding proteins ULBP1 - ULBP4 [40], which also interact with NKG2D.

Viruses are a main target of antigen presentation by MHC class I molecules. As most viral genomes are characterised by a high mutation rate and harbour genes involved in immune evasion, MHC class I genes have to keep pace with

viral evolution. Thus, class I genes evolve rapidly and are a paradigm of positive Darwinian selection [41,42], driven by pathogenic challenge and resulting in this extraordinary high polymorphism. The three-dimensional model of MHC class I molecules [43-45] solved the significance of this polymorphism. It could be demonstrated by Hughes and Nei [Ref. 41] that those codons that encode the peptide-binding region (PBR) of class I molecules show a much higher rate of non-synonymous (d_N) compared to synonymous substitutions (d_S). In contrast, neighbouring codons, which do not encode PBR residues, show $d_S > d_N$. This indicates positive selection for PBR codons and purifying selection for non-PBR codons. The group of Hans-Georg Rammensee experimentally confirmed these important findings in the understanding of MHC class I molecules' function and the significance of their polymorphism in a smart study. By pool sequencing of eluted peptides it could be shown that alleles of MHC class I molecules differ in the spectrum of bound peptides [46,47], giving a plausible explanation of the meaning of MHC polymorphism. Moreover, these data illustrated the selective advantage of MHC heterozygous individuals ('heterozygote advantage'), which was postulated 30 years ago by Doherty and Zinkernagel [Ref. 48] and confirmed by others [49,50].

Receptors of NK Cells

NK cells play an essential role in the elimination of virally infected cells and tumour cells. As part of the innate immune system these lymphocytes can be rapidly activated and are able to kill target cells. This is usually accomplished

by interaction of a variety of receptors and ligands, which are expressed on the surface of NK cells and target cells, respectively. Various NK cell receptors have been identified and I will focus here on those that interact with MHC class I ligands. NK cell receptors are germline encoded and do not undergo somatic recombination such as B and T lymphocyte receptor genes. Gene transcription is epigenetically controlled, leading to clonally distributed expression of NK cell receptors [51,52]. They are classified according to their function, being either an inhibitory or an activating receptor. Genes encoding both inhibitory and activating NK receptors are clustered in two gene complexes, the natural killer complex (*NKC*) and the leukocyte receptor complex (*LRC*), which map to human chromosomes 12p13.1 [53] and 19q13.4 [54], respectively. The *NKC* encodes type II transmembrane, C-type lectin-like receptors, whereas the NK cell receptor genes of the *LRC* belong to the immunoglobulin (Ig) superfamily [55].

The human *NKC* contains the *KLRD1* (*CD94*), *KLRC1* to *KLRC4* (*NGK2A*, *NGK2C*, *NGK2E*, *NGK2F*), and *KLRK1* (*NGK2D*) NK cell receptor genes. A further gene, *KLRA1* (*LY49L*), is inactivated in humans and belongs to the *Ly49* gene family, which is substantially enlarged in mice [56] and rats [57]. The *CD94* molecule forms heterodimers with *NGK2A*, *NGK2C*, and *NGK2E* [58], while the function of *NGK2F* is still unknown [59]. The cytoplasmic region of the *NGK2A* protein harbours two immunoreceptor tyrosine-based inhibitory motifs (ITIM). *NGK2C* and *NGK2E* represent activating NK cell receptors due to the lack of ITIMs and presence of a positively charged amino acid in the transmembrane region, which is necessary for the interaction with the signalling adaptor molecule DAP12. *NGK2D* forms homodimers and represents an activating receptor. The *CD94* protein does not contain such functionally relevant motifs or amino acids. The *CD94* and *NGK2D* genes are monomorphic in the primate species tested, except for the orangutan where polymorphisms have been reported for the

CD94 gene [60]. For the activating receptor genes *NGK2C* allelic polymorphism have been identified in primates [61-63]. Although the general genomic organisation of the *CD94* to *LY49L* interval appears to be conserved in primates, particularly the activating receptor genes appear to be subject of duplications (Fig. 2). For example the chimpanzee *NGK2C* gene is duplicated [62] and in rhesus macaques, *NGK2C* is at least duplicated (own unpublished data) and several sequences have been identified that appear to be derived from duplications of the *NGK2F* locus [61,64]. In the orangutan, a gene has been identified that is equally related to human *NGK2C* and *NGK2E* and is thus designated *NGK2CE* [60]. The *LY49L* gene is inactivated in humans and chimpanzees due to mutations that affect proper splicing of the transcript [65]. However, in Old World monkeys such as the baboon this gene is expressed and codes for a proper *LY49* protein. Analyses of the splice sites in further primates suggest that *LY49L* is functional as well in gibbon, orangutan and gorilla [65], indicating that the inactivating point mutations have occurred after the split of the gorilla in the lineage leading to human and chimpanzees.

The human *LRC* contains the killer immunoglobulin-like receptor (*KIR*) genes, which code for type I transmembrane receptors with a variable number of Ig domains. The *KIR* proteins are composed of one, two or three extracellular Ig domains, a transmembrane region and a cytoplasmic tail. A short cytoplasmic tail is usually associated with the presence of a positively charged amino acid in the transmembrane region and identifies this receptor as an activating *KIR*. Inhibitory *KIRs* are characterised by long cytoplasmic tails that harbour ITIMs. Accordingly, the nomenclature of *KIR* genes considers these characteristics [66], e. g. the *KIR3DL1* gene codes for an inhibitory receptor with three Ig domains and the product of the *KIR2DS1* gene represents an activating receptor with two Ig domains (D= domain, L= long, S= short; see <http://www.ebi.ac.uk/ipd/kir/nomenclature.html>). Up to 16 human *KIR* genes have been described so far. A

Fig. (2). Presence of *NKC* genes in primate species.

hallmark of *KIR* genes is their extensive polymorphism [67]. Currently, 3 to 42 alleles of the various *KIR* genes have been identified in the human population (for individual *KIR* gene allele numbers see <http://www.ebi.ac.uk/ipd/kir/stats.html>). Notably, human *KIR* haplotypes vary considerably in gene content [68-70] and have been classified into group A and B haplotypes [68,71] based on the number of activating *KIR* genes present on the haplotype (Fig. 3). Due to small and homologous intergenic sequences, *KIR* genes underlie duplications and deletions by non-reciprocal recombination [71], with significant differences in *KIR* genotype frequencies between different ethnic populations [72]. As a result of this extensive genetic diversity, unrelated individuals usually differ in their *KIR* genotype [68,73,74]. Furthermore, as a consequence of differential gene content individuals tend to have a more "inhibitory" *KIR* genotype (e. g. group A haplotype homozygotes) or a more "activating" *KIR* genotype (group B haplotype homozygotes). Such differences are associated with functional effects and disease associations as will be discussed below.

KIR genes have been also identified in non-human primate species such as chimpanzee [75], bonobo [76], gorilla [77], orangutan [60], rhesus macaques [61,78], African green monkey [79] and in common marmoset monkeys [80]. Comparisons between human and non-human primate *KIR* genes revealed rapid evolution of these NK cell receptors: the more distantly related two primate species are, the less orthologous *KIR* genes are found. For example, the only orthologous *KIR* gene of humans and rhesus macaques are *KIR2DL4* and the recently identified *KIR3DLX* (also designated *KIR3DL0*) gene, which maps between the two leuko-

cyte immunoglobulin-like receptor (*LILR*) gene clusters (Fig. 3) and is present in all primate species tested [80]. Similar to humans, non-human primate *KIR* haplotypes differ in gene content [78] and diversity of individual *KIR* genes is generated by domain shuffling [77,79].

Interaction of NK Receptors and MHC Class I Molecules

NK cells scan other cells for the presence of MHC class I molecules. Engagement of inhibitory NK receptors with MHC class I ligands on the target cell inhibits NK cells. Viral infection or malignant transformation often lead to down-regulation of MHC class I molecules and to induced expression of the stress molecules MIC and ULBP [81]. Thus, infection or transformation abrogates inhibitory NK cell signalling and engages interactions of certain MHC class I molecules with activating NK cell receptors, which results in killing of the cell and production of pro-inflammatory cytokines.

The interaction of NK cell receptors and their MHC class I ligands is specific. For the highly polymorphic HLA-A (alleles A*03/A*11) and HLA-B molecules (only alleles carrying the Bw4 serological epitope) specific binding could be demonstrated to *KIR3DL2* and *KIR3DL1*, respectively [82,83]. HLA-C alleles can be grouped according to a functional dimorphism at amino acid position 80: group 1 HLA-C allotypes (HLA-C1) have an asparagine residue and group 2 allotypes (HLA-C2) exhibit a lysine residue at this very position [84]. Both groups bind to inhibitory KIRs, but the dimorphism changes the specificity for KIR molecules: HLA-C1 molecules interact with *KIR2DL2* and *KIR2DL3*,

Fig. (3). Schematic genomic organisation of the LRC on human chromosome 19q13.4. A detailed map of the *KIR* gene cluster is shown for a group A and a group B *KIR* haplotype. Filled and hatched boxes indicate inhibitory and activating *KIR* genes, respectively. *KIR* pseudogenes are shown by the Greek letter psi.

whereas HLA-C2 allotypes bind to KIR2DL1. It could be shown that KIR2DL1, KIR2DL2 and KIR2DL3 molecules bind their cognate HLA-C ligands with different strengths [85]. In contrast to inhibitory KIRs, the HLA-specificity of activating KIRs is less clear. Activating KIRs arose by duplication from inhibitory KIRs [86] and, therefore, pairs of inhibitory and activating molecules show a high degree of similarity. However, despite this similarity, activating KIRs show a much lower strength of interaction with MHC class I ligands than their inhibitory counterparts. Interestingly, the activating KIR2DS1 receptor could be shown recently to interact with the HLA-C ligand in a peptide-dependent (HLA-C-presented peptides) manner upon infection with the Epstein-Barr-Virus [87], emphasising the importance of activating KIRs in immunity to infections.

A further interesting interaction is seen between HLA-E and CD94/NKG2 heterodimers. HLA-E presents peptides that are derived from leader sequences of other MHC class I molecules [88] and binds to CD94/NKG2 heterodimers [89]. This is thought to function as a monitoring of MHC class I protein expression, which might become down-regulated upon viral infection. Unavailability of class I leader peptides would then result in loss of HLA-E expression on the surface of the infected cell and in abrogation of NKG2A/CD94-mediated inhibitory signalling in the scanning NK cell.

Certain combinations of the *HLA* and *KIR* genotypes are associated with more or less inhibition of NK cells, which is thought to predispose the carrier to certain diseases [90]. Hiby and colleagues [Ref. 91] reported an interesting finding. They observed certain combinations of maternal *KIR* haplotype and fetal *HLA-C* genotype in patients suffering from preeclampsia, a serious complication in pregnancy. NK cells are frequently found in the placenta and provide cytokines that are essential for the establishment of fetal blood supply [92]. Homozygosity for group A *KIR* haplotypes (see Fig. 3) and two copies of a fetal *HLA-C2* type (see above) obviously results in strong inhibition of placental NK cells and, thus, in low production of cytokines and higher risk of fetal loss. Therefore, certain combinations of *KIR* and *HLA* genotypes can influence reproductive success. Indeed, such selective pressure likely shaped the genotype frequencies and polymorphism of KIRs and HLA ligands in human populations. For example a high frequency of group A *KIR* haplotype homozygosity and a low frequency of *HLA-C2* types are found in the Japanese and an inverse correlation is evident in the Australian Aborigine population [91].

Considerable genomic differences of MHC and NK cell receptor loci are apparent in primates. These genes evolve rapidly due to high selective pressure to combat infections. Even within a primate species large haplotypic differences are encountered with respect to allelic diversity and gene content. Certain genotypes and particularly combinations of different genotypes can predispose the host to infectious and autoimmune diseases and can severely influence reproductive success. Moreover, the various genotype combinations can have opposing effects, for example activating *KIR* haplotypes have a beneficial effect with respect to infectious diseases and reproduction, but are associated with susceptibility to develop an autoimmune disease. So far, we are just at the beginning of understanding all implications of these complex interactions.

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