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Decreased NK Cell Frequency and Function Is Associated with Increased Risk of KIR3DL Allele Polymorphism in Simian Immunodeficiency Virus-Infected Rhesus Macaques with High Viral Loads[1]

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Abstract

NK cells have been established as an important effector of innate immunity in a variety of viral infections. In HIV-1 infection in humans, alterations of NK cell function, frequency, and expression of various NK receptors have been reported to be associated with differential dynamics of disease progression. Expression of certain alleles of KIR3DL and KIR3DS receptors on NK cells was shown to correlate with levels of virus replication. In the SIV-infected rhesus macaque (RM) model of AIDS, several families of killer inhibitory Ig-related receptors (KIR receptors) corresponding to their human counterparts have been characterized, but only at the level of individual sequence variants. Here we define 14 different alleles of KIR3DL expressed among 38 SIV-infected RM, characterized by either high or low levels of SIV replication, by analyzing multiple sequences from individual animals and show an unequal distribution of certain alleles in these cohorts. High levels of SIV replication were associated with significant increases in KIR3DL mRNA levels in addition to decreases in both the frequency and function of NK cells in these animals. The higher frequency of inheritance of two KIR3DL alleles characterized by a single nucleotide polymorphism 159 H/Q was associated with RM that exhibited high plasma viral load. This data for the first time defines multiple alleles of KIR3DL in RM and shows an association between virus control, NK cell function and genetic polymorphisms of KIR receptors.

Our concepts of the role NK cells play in the orchestration of immune responses has changed markedly due to findings from a number of relatively recent studies, which suggest a more sophisticated role for the NK cells than previously assumed. Thus, NK cells appear to undergo a process similar to negative/positive selection by T cells in that they have been

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shown to undergo “licensing” and “arming” in the bone marrow (education) via the interaction of select inhibitory receptors with MHC class I to acquire full functional maturation (1), a process of inactivation and/or depletion via the interaction of activating receptors following interaction with a high affinity ligand expressed by other bone marrow cells (2, 3), and, finally their ability to execute immunoregulatory (NKreg) functions (4 –7). These distinct sets of functions are coordinated by the expression of a variety of both “inhibitory” and “activating” receptors (Fig. 1). One of these receptor families—the killer inhibitory Ig-like receptors (KIRs)[3]— has recently gained much attention, as increasing amount of data showed association of specific KIR variants with resistance or susceptibility to various diseases (8, 9). KIR molecules represent multigene family encoding receptors displaying different functional characteristics and ligand specificities which recognize certain MHC class I molecules (10). These receptors can relay either activation or inhibitory signals to NK cells (11). In general, the KIRs are expressed with either 1 to 3 extracellular Ig-like domains (D0 to D2), a transmembrane domain and short (S) or long (L) cytoplasmic tails, which contain ITIM motifs or which can pair with ITAM-containing adaptor molecules such as DAP12 (12).

The finding that the major target of both HIV and SIV are the gut-associated lymphoid tissues (GALT) and that the quality and quantity of gut-associated pathology that occurs during the acute infection period sets the stage for the kinetics of disease progression (13, 14), has prompted a renewed interest on defining the role of the various cell lineages that could potentially participate in the anti-virus defense during these periods of acute infection. This has led to increased interest in understanding the contribution of cells of the innate immune system including the role of the NK cell lineage. Several recent studies have shown a correlation of various NK cell genetic and functional characteristics with the course of AIDS progression (15–17). Thus HIV-1 replication in patients during both acute and chronic phases of HIV-1 infection was found to correlate with the extent of NK cell activity and to affect the dynamics of the NK cell subpopulations, leading to the dominance of an anergic NK cell subtype during the chronic phase of the infection (18–20). More interestingly it was shown that expression of certain families of KIR receptors and specific genetic KIR and HLA class I combinations correlate with a protection from the development of the HIV-1 induced disease. Thus increased expression of KIR2DL3 on NK cells correlated inversely with disease severity in HIV-1-infected children (21). Large population genetic studies in HIV-1-infected patients have shown a positive correlation between coexpression of various KIR3DL1 or KIR3DS1 alleles and certain HLA class I alleles with the delayed progression toward AIDS (22, 23). From the mechanistic point of view, it was shown that KIR3DS1 expressing NK cells inhibit HIV-1 replication in target cells and preferentially lyse HIV-1-infected target cells that express HLA-B Bw4-80I (24).

Our laboratory has been involved with studies of the mechanisms of pathogenesis of lentivirus infection using the simian immunodeficiency virus (SIV) infected non-human primate model (25–27). We and others have shown that, similar to HIV infection in humans, there are phenotypic changes and functional impairment in the NK cell population that occur

[3]Abbreviations used in this paper: KIR, killer inhibitory Ig-like receptor; RM, rhesus macaque; VL, viral load; HVL, high VL; LVL, low VL; SNP, single nucleotide polymorphism.

during both acute and chronic phases of SIV infection in rhesus macaques (RMs) (28, 29). The level of NK cell responses has also been shown to indirectly correlate with the extent of CNS injury in the SIV model of CNS disease (30). Five distinct families of KIR receptors have been identified in RMs (31) and genomic loci coding for these receptors have been localized in RM to chromosome 19 (32, 33).

As an initial attempt to characterize the role of KIR genes in influencing disease course in RM we focused our studies on the role of the KIR3DL family on two cohorts of SIV_{mac251}-infected RM with distinct rates of disease progression/virus control. The aim of this study was to define alleles/genomic polymorphisms of KIR3DL receptors in RM and delineate potential correlation between these alleles, levels of NK cell responses and control of virus replication. Results of our studies using multiple sequence expression analysis demonstrate that these RM express a total of 14 different alleles of the KIR3DL family and show their unequal distribution between animals which exhibit good vs poor control of SIV replication.

Materials and Methods

Experimental animals

The peripheral blood samples were obtained from normal healthy adult RM (*Macaca mulatta; n* = 24) and SIV-infected RM (*n* = 38) housed at the Yerkes Regional Primate Research Center of Emory University. All animals were maintained according to the guidelines of the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council and the Health and Human Services guidelines Guide for the Care and Use of Laboratory Animals. SIV-infected RM (infected with SIV_{mac251} or SIV_{mac239}) were separated into two cohorts based on the levels of plasma viral load (VL) at the chronic stage of infection or disease progression that remained essentially similar for at least 3 sequential months: 1) a cohort of high viral load (HVL) monkeys (*n* = 20), defined as animals with VL > 10^6 virus copies/ml of plasma (range between 1.2×10^6 and 4×10^7; average 10^7 copies/ml), or monkeys who progressed toward AIDS-like disease within 8 mo; and 2) a cohort of low viral load (LVL) monkeys (*n* = 18), defined as animals with VL < 10^5 virus copies/ml of plasma (range between 3×10^2 and 7×10^4; average 10^3 copies/ml). Samples were obtained from the same animals pre-and post-SIV infection from a majority of these animals. Flow cytometric analysis of NK cells was performed as previously described (28). MHC class I (Mamu) typing for Mamu A.01, B.01, B.08, and B.17 was performed as previously described (34), except for the Mamu B. 17, where primers 5-GGA TTA CAT CGC CCT GAA CGG T-3 and 5-GCC TGG TCT CCA CAA ACT CT-3 were used.

Isolation of NKG2A$^+$ NK cells

PBMC were isolated using lymphocyte separation medium (Cellgro) and first depleted of $CD3^+$ T cells by incubating with unconjugated anti-CD3 mAb (1 μg/million cells, clone FN-18; BioSource) followed by incubation with anti-mouse Ig-conjugated magnetic beads (Cellection kit; Invitrogen). Bead-bound T cells were discarded and remaining $CD3^+$ lymphocytes were incubated with unconjugated anti-NKG2A mAb (1 μg/million cells, clone

Z199; Beckman Coulter) followed by positive selection with anti-mouse Ig-conjugated magnetic beads (Cellection kit). The purity of NKG2A$^+$ cell population was always >90%.

Culturing and labeling of K562 target cells

A culture of K562 cells was maintained in 10% RPMI 1640 with 10% FCS, 2 mM glutamine, and 50 μg/ml gentamicin. The cell suspension was pelleted at 1000 rpm for 5 min and resuspended at a concentration of 0.5 million cells/ml in 10% RPMI 1640. From this culture, 2 ml was added to 8 ml of fresh 10% RPMI 1640 (1:5), and [^{3}H]thymidine was added at a final concentration of 1 μCi/ml. The culture was incubated overnight at 37°C, 5% CO_2. The following day, K562 cells were counted, washed with 10% RPMI 1640 and resuspended at a concentration of 100,000 cells/ml. The washed cells were allowed to sit for 1 h at 37°C, 5% CO_2 until use in the JAM assay as previously described (35).

Cloning of KIR3DL sequences

Total RNA was isolated from highly enriched population of NKG2A$^+$ NK cells using the RNeasy kit (Qiagen) and cDNA was synthesized using the ProtoScript cDNA synthesis kit (New England Biolabs). KIR3DL sequences were amplified from all animals using primer pairs as follows: 5-CATACACGGGTGGTCAGGACAAGA-3 and 5-CAGCGATGCAG GAGAAAGAAGAAG-3. The primers were designed in the conserved regions of the KIR3DL to amplify all known sequence variants (31) and to cover a majority of the KIR3DL sequence. The resulting PCR products were size-purified and cloned into the pGEM-T vector (Promega). Five KIR3DL sequence-containing colonies from each cloning/transformation were picked up and clones were sequenced using SP6 and T7. Additional KIR3DL sequences (covering sequences that include “tail” regions of select alleles) were amplified using primers 5-TGGCGATCAGG/AGTCA CAGGAGTCC-3 and 5-TCAAGGCCTGACGGTGGTGCTCAT-3, and cloned as described above. Sequences were analyzed using the DNASTAR analysis package.

Real-time PCR quantification of KIR3DL

cDNA samples from NKG2A$^+$ NK cell samples from RM pre- and post-SIV infection were subjected to real-time PCR in an iCycler (Bio-Rad) and SYBR-Green fluorescence quantification was performed using primers for KIR3DL cDNA: 5-ACCCACTGAACCAAGCTCCAAAAC-3 and 5-CG GTGATCCAACTGTGCGTATGTC-3. As a control, an amplification of the GAPDH fragment was performed using the primers 5-ACCACCAT GGAGAAGGCTGG-3 and 5-CAGTTGGTGGTGCAGGAGGC-3. Parameters of the cycle were 95° for 15 min and 60° for 1 h with subsequent melt-curve analysis, which showed a single T_m peak per sample within the 88–89°C range. The target KIR3DL cDNA quantitation in duplicate samples was then performed by first normalizing the threshold cycle number of the target gene to the GAPDH. The copy numbers of the target gene in each animal post-SIV infection were then expressed relative to the copy numbers obtained from the pre-SIV infection sample from the same animal. Each target sequence and GAPDH control was quantitated from two independent cDNA preparations from each sample/animal and the resulting relative quantitation is expressed as an average of two measurements.

TaqMan analysis of genomic DNA

DNA was isolated from purified NK cell samples using the Wizard Genomic DNA purification kit (Promega). A TaqMan Allelic Discrimination assay (Applied Biosystems) was custom designed to investigate the single nucleotide polymorphism (SNP) at the KIR3DL position 159. A pair of PCR primers (KIR-138F: 5-GAGACGG TCACCCTACAATGTTC-3 and KIR-138R: 5-AATAGTTGGCCTGGGAACCC-3) and two fluores-cent probes (Reporter1-VIC: TTCTTTCTGCAAAGTGAG and Reporter2-FAM: TTCTTTCTGCACAGTGAG) were used in an SNP assay that was conducted using a 7900HT fast real-time PCR system (Applied Bio-systems). Each of the 5 μl of PCRs consisted of 10 ng of genomic DNA, 2.5 μl of TaqMan genotyping mastermix, and 0.125 μl of primer/probe mix. The PCR program was set for 10 min at 95°C, followed by 45 cycles of 15 sec at 92°C and 1 min at 60°C. Post-PCR, the assay endpoint was read, and the intensities of the two reporters were used to compute the relative abundance, therefore the allelic composition of the SNP under interrogation. The analysis was performed using the SDS v2.1 software (Applied Biosystems).

Results

Sequence analysis and KIR3DL allele designation in RM

Several reports have previously shown an association of certain KIR alleles, especially those that belong to the KIR3DL and KIR3DS family, with distinct course of disease in HIV-1 infected humans (23, 36, 37). Although there are sequence data available for KIR-related genes in non-human primates from previous studies (31–33, 38), these data represent single sequences from individual animals and therefore do not compile a database large enough to identify sequence patterns or alleles. Because changes in the expression of KIR molecules have been previously shown to be associated with differences of disease course in both HIV-1 infected humans and SIV infection in RM (21, 29), we first assessed whether SIV infection induces any global difference in KIR3DL expression in our HVL and LVL cohorts. Thus purified NK cells from six animals from each of two the cohorts were tested by real-time PCR quantification for the levels of KIR3DL expression both pre- and post-SIV infection. The results in Fig. 2 (expressed as fold change in each animal post-SIV infection vs the pre-SIV infection baseline) show that although expression of KIR3DL in the LVL animals stays at approximately the same level postinfection (mean 1.7-fold increase), the HVL animals exhibit more than a 5-fold increase in KIR3DL expression after SIV infection (mean 5.7-fold; $p < 0.02$). These data prompted us to conduct more detailed studies of KIR3DL.

To perform studies aimed at the identification of KIR3DL alleles that might be potentially associated with a difference in immune responses and subsequently altered disease course in the RM model of AIDS, we first aimed to build a database and define KIR3DL alleles by analyzing multiple KIR3DL sequences expressed by NK cells from each of the 38 animals in our two cohorts. Sequences representing D0, D1, D2, and the transmembrane domains of the KIR3DL molecule were amplified by PCR from cDNA prepared from highly purified NKG2a$^+$ cells from each animal and five clones containing KIR3DL-related sequences were subsequently sequenced. Table I shows that clones from a majority of the animals yielded

various KIR3DL variants, except for 4 animals that did not yield any KIR3DL-related products using our primer pair. A majority of these sequences represented full-length or wild-type KIR3DL sequences. However in several animals, only splice variants related to the splice variants 2, 5, and 7, as defined by Hershberger et al. (31), could be detected. In addition to the full-length sequences, which exhibited a relatively higher degree of similarity to some of the sequences previously published, new sequence variants were obtained from several animals that showed higher degree of diversity and were therefore designated as new variants A through N.

To identify sequence patterns among all the individual sequences, that would allow us to define several closely related groups, or alleles, the sequences were first translated, to disregard those nucleotide variations that do not result in a change of aminoacids and therefore in a potential structural change of the receptor. The protein sequences were subsequently grouped based on the phylogenetic distance in several steps. In the first step, sequences from each animal were analyzed separately and in most animals this led to the establishment of one or two groups of closely related sequences within the animal (>98% similarity). These would presumably identify one or two alleles of the gene being expressed in each animal. In a few animals, the clones yielded sequences that segregated into more than two different groups. Consensus sequences were generated in each animal for each of the presumed alleles and these were subsequently compared phylogenetically between the animals (Fig. 3). This led to the identification of a total of 14 primary allelic sequence groups within these animals (Fig. 4; GenBank accession numbers FJ562108 through FJ562121). A majority of these allelic groups is characterized by close phylogenetic relationship/sequence identity (>98%) between sequences of clones from at least two different animals. Only alleles 4 and 9 were each defined by multiple clones within only one animal. A few sequences represented individual variants not related to any of the other sequences and were not therefore designated as individual alleles. Since these comparisons were performed at the protein level by generating consensus protein sequences through several steps, which could potentially mask DNA variations in some individual sequences by a majority, a reverse analysis was subsequently performed, whereby the untranslated DNA sequences were grouped into allelic groups defined above. The analysis showed that both the protein and the DNA sequences formed identical phylogenetic patterns, segregating into 14 groups, each of which was defined by phylogenetic distance/sequence similarity (>98%; data not shown). Most of the newly identified variant sequences—variants A through N (Fig. 5)—were present in single clones only, fairly divergent from each other and therefore treated as separate variant sequences. The exception was variant A, which was found in five different animals and showed a relatively high degree of homology and was therefore grouped with the 14 common alleles as an allelic variant. Most of the other newly identified variants showed more or less extensive deletions in various parts of the sequence (Fig. 5, *B*–*E*). These deletions led to a frameshift and premature stop-codons in these variants. The exceptions were the variants B and C that showed relatively large insertions within the D2 region of the sequence (Fig. 5*A*). Results of the sequence alignment of the alleles showed varying degrees of diversity between the groups with all the extracellular domains affected to a similar extent. The cysteine residues in each of these domains, necessary for the formation of disulfide bonds, are, however, preserved in each allelic group.

The assignment of the alleles based on the cloned sequences back to individual animals is shown in Table II. The splice variant 2 sequences, detected in several animals, showed a high degree of homology with one of the respective full length alleles detected in each animal, except for the animal mm19, where it was most closely related to allele 1. In those animals, where only splice variants were obtained, the majority of the clones were closely related to allele 8. Table II also shows MHC class I (Mamu) positivity for 4 alleles: Mamu A.01, B.01, B.08 and B.17.

Taken together, the sequence analysis of multiple KIR3DL clones in each RM from a cohort of 38 monkeys allowed us to identify 14 basic KIR3DL alleles in RMs and assign these alleles back to individual animals.

Allelic distribution of KIR3DL in RM with high and low SIV VL

Based on the compiled database of sequences from individual animals and allelic assignments, we further analyzed these data in efforts to determine the frequency of the expression of these KIR3DL alleles among the 2 cohorts of SIV infected RM. Table III shows the frequencies of the alleles within all the animals (ALL) and in the two cohorts separately (HVL, LVL). Most alleles showed a relatively low frequency, occurring only in 5–20% of the 28 animals, from which the full-length sequences were obtained, and the distributions of these alleles between the cohorts were relatively comparable. The four animals that did not yield any KIR3DL sequences (mm 22, 25, 28 and 31) were equally distributed between the HVL and LVL groups. Interestingly, two alleles—8 and 13—were relatively more prevalent and were detected in 25% and 32% of the animals in general, respectively. These two alleles also showed differential distribution between the HVL and LVL cohorts. Thus, allele 8 was more prevalent in the LVL cohort (38% vs 13% in LVL vs HVL, respectively), but when considering additional sequence information derived from the splice variant 2 clones, where a majority of these sequences were close to allele 8, the frequencies of allele 8 between the 2 cohorts became comparable (data not shown). The second allele, that showed a relatively high prevalence, allele 13, showed the opposite trend as it was ~3 times more prevalent in the HVL cohort (47% vs 15%, HVL vs LVL, respectively). Interestingly, a closer examination of the sequences of individual alleles has shown, that allele 13 and allele 14 share a set of relatively closely spaced amino acid substitutions predominantly in the D1 domain, that are not present in any other allele (the “Q-KxSV-MGP-A” motif). When we re-analyzed the allelic frequency data to determine, which animals express KIR3DL bearing this motif, i.e., either allele 13 or allele 14, the added two-allele frequency was even more skewed toward the HVL cohort (60% vs 15% in HVL and LVL, respectively). We also tested animals in both cohorts for the expression of 4 MHC class I (Mamu) haplotypes—A.01, B.01, B.08 and B.17 (Table II). The most frequent allele, Mamu A.01 was present in 24% animals overall and was about twice as much prevalent in the LVL than the HVL cohort (33% vs 15%, respectively). Interestingly, however, only 1 of the 6 Mamu A.01 positive LVL animals was found to express alleles 13/14, while all 3 Mamu A.01 positive HVL animals were found expressing alleles 13/14.

These data suggest that a potentially relatively strong association exists between the expression of KIR alleles 13 and 14 and poor control of SIV replication. To ensure that the

allelic sequences defined above constitute domains of KIR3DL with appropriate cytoplasmic tail, we have cloned and sequenced the remaining 3′ end (or tail) sequences for the majority of these alleles. Fig. 6 shows the alignment of these sequences for alleles 13, 14, 8 and published human KIR3DL sequence (NM_013289) indicating that the allelic D0–D2 sequences defined in this study indeed represent domains of KIR3DL variants with intact "long" cytoplasmic tail. One has to keep in mind, however, that the allelic frequencies were calculated based on data derived by expression cloning. This approach allowed for the compiling of sequence data, allelic designation and some knowledge as to what alleles are being expressed at the level sufficient for the detection by the PCR/cloning approach. However, it is possible, that we did not detect some of the less prevalent sequences by this approach and it also did not allow us to investigate the total number of alleles at the genomic level. To further investigate the prevalence of alleles 13 and 14 in the two cohorts, we designed a TaqMan assay for the analysis of genomic DNA samples from the animals belonging to the two cohorts. We took advantage of the unique position 159H-Q amino acid substitution based on the unique SNP in the alleles 13 and 14, which was always a part of the Q-KxSV-MGP-A motif characteristic for these 2 alleles, but distinguished them from all the other alleles detected. When we genotyped all the animals for this particular SNP (Table IV), the data showed that the animals were either H/H homozygotes or H/Q heterozygotes, with the latter being more prevalent overall. Interestingly, a majority (85%) of the HVL animals were H/Q heterozygotes, while only 43% of the LVL animals were H/Q heterozygotes. Taken together, both the expression analysis (performed by the cloning/sequencing approach) and the genomic analysis showed a significantly higher prevalence of KIR3DL alleles harboring the 159H/Q SNP within the HVL animal cohort.

Decreased NK cell frequency and function is associated with poor control of SIV in RMs

Previous data from HIV-1 infection in humans suggested that there is an inverse association between viral load/replication during chronic infection and both NK cell frequency and function (18, 20). Similarly, data from several laboratories, including ours, have suggested that the pathogenic SIV infection in RMs is associated with a decrease of NK lytic function and cytokine secretion (28, 29, 39). Our analysis above showed relatively unequal distribution of the newly defined KIR3DL alleles and differential levels of KIR3DL expression between the animals exhibiting high vs low SIV replication. To further define, whether the differences in virus control were associated with altered NK cell function, the lytic NK cell activity was analyzed in the two different cohorts of chronically SIV infected RMs and compared with the cohort of SIV naive healthy animals. Thus, we analyzed the cytolytic activity of the NK cells in the majority of the animals exhibiting low viral load (LVL; $<10^5$ virus copies/ml of plasma; $n = 10$) and animals exhibiting high viral load (HVL; $>10^6$ virus copies/ml of plasma; $n = 14$) and compared these to the levels obtained on samples from a group of healthy uninfected RM ($n = 24$). The data show (Fig. 7*A*) that while the mean cytolytic activity levels obtained from the NK cells from the LVL animals are indistinguishable from those obtained from uninfected healthy macaques (680 vs 650 lytic units per 10^6 cells, respectively), the animals in the HVL group exhibit markedly and significantly diminished mean NK cell mediated lytic activity (173 lytic units per 10^6 cells; $p < 10^{-13}$ from both the uninfected and LVL RMs).

In addition, we also aimed to analyze the effect of SIV infection on NK cell frequencies in the two cohorts of animals. NK cell frequencies were established by flow cytometric analysis of 2–3 sequential PBMC samples pre-SIV infection and the frequencies were compared with the frequencies obtained in a similar fashion from the same animals during the chronic phase of infection. For this flow-cytometric analysis, the frequency of $CD3^-$ $CD8^+$ population was analyzed as a correlate of the frequency of NK cells. This population, as shown previously, in RMs contains a large majority (>80%) of $NKG2A^+$ cells and the total $NKG2A^+$ population within the lymphocyte population was found to be similar to the percentage of $CD3^-$ $CD8^+$ (28). The comparison was then made for each animal as ratio post vs pre infection and mean ratios were obtained within each cohort (Fig. 7*B*). The data show that while the trend in LVL animals was to maintain the frequency of CD3-$CD8^+$ cells at the pre-infection levels (average relative frequency ~1), the HVL animals exhibited on average a 50% decrease in the frequency, and this difference between the post and pre-SIV frequencies was highly significant ($p < 10^{-6}$).

Taken together, these data show, that high virus load/poor control of the virus replication in the HVL animals is associated with significant decreases of both the frequency of NK cells and NK-associated lytic activity. Conversely, the SIV infected monkeys that control virus replication, maintain NK cell frequencies at the pre-infection levels and the NK lytic activity in these animals is indistinguishable from uninfected healthy controls.

Discussion

In this study we report for the first time an extensive analysis of a single KIR family in a relatively large cohort of RMs of the Indian origin with the aim to identify individual alleles and subsequently investigate potential correlation of these alleles to the course of disease due to SIV infection. Several previous studies have reported data showing a general characterization of KIR families in RMs and other primates both at the expression and genomic levels (31–33, 38). In the most extensive study to date, Hershberger et al. (31) cloned and sequenced KIR related sequences from RM (several clones per animal) and identified 5 KIR families based on their structure and homology to human KIRs—KIR1D, KIR2DL4, KIR2DL5, KIR3DL, and KIR3DH. Some of these families are represented by several sequence and splice variants, as they were found in individual animals. We chose a different approach and chose to focus on one KIR family—KIR3DL—with the aim to perform analysis from a relatively larger population of RM and define sequence variation that are common in the population and can be defined as alleles. From the standpoint of SIV infection, the choice of one of the KIR3D families was obvious, as the expression of certain alleles of KIR3DL1 and KIR3DS in humans, together with certain MHC-I haplotypes, was shown to have a significant effect on NK cell function or correlation with distinct HIV-1 disease progression (22–24). To compile a sufficient database that would allow us to perform similar analyses in the non-human primate model of AIDS, we cloned and sequenced KIR3DL related sequences from purified NK cells from a total of 38 animals, five KIR3DL positive sequences from each, which therefore represents a relatively large expression screen of NK-specific KIR3DL receptors. Only 4/38 animals (10.5%) failed to yield any KIR3DL related sequences. Subsequently we utilized a multistep approach to define phylogenetically close and common patterns among these sequences that would allow us to define consensus

sequences representing full-length KIR3DL alleles present in the population. By this approach we defined 14 different alleles, where each of them represents multiple sequences from individual animals and 12 of them are present in 2 animals or more. Alignment and phylogenetic comparison of our alleles to previously published KIR3DL molecules (31) showed that some of our alleles are close to one of the previously published KIR3DL variants—e.g., KIR3DL8/Allele8, KIR3DL2/Allele2, KIR3DL11/Allele11, KIR3DL7/Allele10 and KIR3DL10/Allele6 (Fig. 8). However, other newly defined allelic sequences are rather dissimilar from the published variants—e.g., alleles 4 and 12—and the alleles 13 and 14 represent an entirely new set of KIR3DL sequences, as they branch separately and none of the previously published sequences contain the Q-KxSV-MGP motif in the D1 domain. It is interesting, that the previous cloning efforts did not pick up these sequences related to our alleles 13 and 14 before, as we found them to be the most prevalent in the population overall (32% overall). One potential explanation could be, that we used purified, or highly enriched population of NK cells and therefore we were detecting only KIR3DL transcripts expressed by NK cells from a relatively large cohort. It is possible that since the study by Hershberger used cDNA isolated from total PBMC, these transcripts could have been masked by the relative abundancy of other KIR transcripts from CD8 T cells as well. The other explanation could be that their population was relatively small. The next two most prevalent alleles are the alleles no. 8 and no. 1, which were present in 25% and 18% of the animals, respectively, similar to the findings by Hershberger et al., who detected their mm-KIR3DL1 (our allele 1) and mmKIR3DL8 (our allele 8) in 3 of the 5 animals analyzed. In addition to the full-length sequences, we detected 3 types of previously published splice variants and several new variants. Most of these new variants were present in individual animals, except for variant A, characterized by a 3 amino acid deletion in the D0 domain, that was present in 4 different animals and therefore resembled an “allele”. The remaining variants were characterized by substantial nucleotide deletions and/or insertions that would have a profound effect on the putative amino acid sequence and receptor structure. The assignment of the alleles back to the animals showed that individual animals expressed simultaneously 1 to 3 alleles plus occasionally some new variants.

As stated previously, one of the primary aims of this study was to define KIR3DL alleles that might be associated with a control in the levels of SIV in the HVL and LVL cohorts. Indeed, we were able to define a novel sequence, represented by alleles 13 and 14, that showed a relatively high prevalence in the population altogether, but more interestingly, showed a four fold difference in the frequencies between the HVL and LVL populations at the expression/cloning level, 60% vs 15%, respectively. This was very encouraging, however the full-length sequences were available only from 28 of 38 animals. In addition, while the cloning/sequencing approach on the one hand allowed us to likely obtain the most prevalent sequences expressed by the individual NK cell sample, it was important to investigate the prevalence of this particular allele at the genomic level. Therefore we performed TaqMan analysis directed at the unique SNP 159 H-Q present only in alleles 13 and 14. Results of this analysis showed, that this SNP is present in a H/Q heterozygous form in 70% of the all animals tested, which is ~1.8-fold higher frequency than obtained with expression cloning. This is understandable, since not all the animals possessing this SNP have to express the Q bearing allele. Nevertheless, even at the genomic level, the relative frequencies of the H/Q

heterozygotes were still positively skewed in the HVL cohort (85%) vs the LVL group (43%). Clearly, further analysis of these animals is necessary with regards to identification of the specific MHC class-I allele that is functional in its interaction with a given KIR3DL allele. Such an association has clearly been shown in select cohorts of HIV-1 infected patients such as the association of specific KIR3DL/KIR3DS and specific HLA- class I combinations that appear to confer a clear advantage for the establishment of good control of HIV-1 in humans and NK cell mediated cell lysis (22–24). Similar analysis in rhesus monkeys is difficult at present, since there is a relatively narrow range of reagents available for these studies.

However, several studies have shown, that in HIV-1 infected humans, expression of select KIR3DL/KIR3DS receptors regardless of their respective ligands, or the changes of levels of expression of these receptors is associated with the resistance to infection or more favorable disease course (36, 37, 40, 41). Therefore it is possible that expression of the KIR3DL alleles 13 or 14 is one of the important factors contributing to the observed decline of NK cell function in the HVL group during the chronic phase of infection. In addition, we show in select animals that a poor control of the virus is associated with selective up-regulation of KIR3DL expression at the mRNA level. Although we did not see significant differences in the extent of NK function decrease between allele 13/14 negative and positive animals in the HVL group, it is possible, that a combination of the KIR3DL allelic background, together with the levels of KIR3DL expression and likely also in combination with certain MHC-class I haplotype may represent the major underlying cause of the observed differences in NK cell function and, subsequently, virus control. In other words, while there are likely several mechanisms involved in regulating viral load (which may include levels of activated $CD4^+$ T cells, levels of Tregs, to name a few), the genetic inheritance of KIR3DL allele 13/14 maybe a contributing factor that promotes higher VL. Clearly, these issues will have to be addressed in further studies.

Finally we confirm, that poor control of the virus is strongly associated with a decline of NK cell function in SIV infected RMs. Results from previous studies in both HIV infection of humans and SIV infection of non-human primates have shown that disease progression is associated with various alterations of NK cell function and phenotype. Thus it was observed, that after the acute phase of HIV infection, the ongoing viral replication led to a depletion of cytolytic $CD3^-CD56^{(dim)}CD16^+$ NK cells with a concurrent increase in a subpopulation of anergic NK cells accompanied by reduced NK cell activity (18). This decrease of mature NK cells in viremic HIV positive individuals was shown to be independent of $CD4^+$ T cell counts (20). Long-term-nonprogressor (LTNP) HIV-1 infected patients show higher relative levels of NK cell activity compared with the viremic individuals and late stage HIV disease is characterized by a decrease of cytotoxic NK cells (42, 43). In vitro induced IFN-α-stimulated NK cell activity were shown to be decreased in NK cells from HIV-1 infected patients exhibiting symptoms of disease when compared with asymptomatic patients (44). Similarly in SIV infection of RMs, in vivo administration of IL-12 led to the gradual increase in absolute NK cell numbers and frequency in the animals at early stage of SIV infection, but had no measurable effect in the late stage animals (39). Supernatant fluids from NK cells obtained from patients who were controlling viral replication were far more effective in suppressing HIV-1 replication in vitro than supernatant fluids from viremic

patients, with an inverse correlation between the level of viremia and the ability of NK cells to suppress HIV replication (45). Several mechanisms for the virus mediated NK cell dysfunction were proposed, including direct effects of the exposure of NK cells to HIV-1 derived gp-120 (46) and regulatory effects of virus derived Vpr on cytokine production (47). Interestingly, a comparative study of NK cell function in patients with HIV-2 infection as compared with HIV-1 infection revealed that high $CD4^+$ T cell counts and low levels of plasma viremia in the former was associated with even higher levels of NK cell cytotoxicity than the HIV-1 infected patients (48). We and other have shown that in RMs, similar to humans, NK cells can be characterized by three different subpopulations and that the viremic SIV infection leads to perturbations of NK cell subpopulations and a decrease in NK cell function, which is not present in LTNP animals or apathogenically SIV infected sooty mangabeys (28, 29, 49, 50). Similar to these previous data from HIV and SIV infection, herein we show that effective control of the virus/low viral load is associated with the maintenance of NK cell numbers and function in relatively large cohorts of SIV infected RMs. Conversely, poor control of virus replication is associated with highly significant ~ 3-fold decrease in NK cell frequency. As a measure of NK cell frequency we utilized $CD3^-CD8^+$ population without subsequent characterization of the 3 individual NK phenotypes. However, this population in RMs consists of more than 80% $NKG2A^+$ cells. The frequency of the $CD3^-CD8^+$ cells within the total lymphocyte population was found to be similar to the frequency of the $NKG2A^+$ cells and therefore represents, in our opinion, a reliable measure of NK cells (28). It is possible that the decrease in the frequency of NK cells in the high VL monkeys could be secondary to the down modulation of the NKG2a molecule and subsequent inability to identify these cells using NKG2a as a marker. Phenotypically, however, our strategy for calculating the frequency and absolute numbers of NK cells is based on first using a wider gate (to include large size NK cells), gating out $CD14^+$ (monocytes/macrophages) and $CD20^+$ (B cells) and then gating on $CD3^-/CD8^+$ cells and analyzing not only $NKG2a^+$ cells but also cells which are $CD3^-CD8^+$ and are also either $CD16^+/CD56^-$ (the major cytolytic NK cell subset), $CD16^-/CD56^+$, $CD16^+/CD56^-$ and $CD16^+/CD56^+$. Thus, if there is down modulation of NKG2a, the use of these additional markers allows us to obtain a more detailed picture of the effect of SIV on NK cells and their subsets. Down modulation of NKG2a in terms of relative density is noted in SIV infected RM but the relative levels are of sufficient density to be readily seen by flow analysis and to be picked up using immuno-beads. Therefore, while it is still possible that there are functional NK cells that cannot be accounted for using these reagents and strategies (because they downmodulate all the routinely utilized markers along with NKG2a), at this stage these markers—including NKG2a—are considered the most optimal method for defining NK cells and its subsets, and the data need to be interpreted within this context. Interestingly also, the pre-infection frequency of NK cells was slightly (~1.5-fold), but significantly higher in the cohort, in which the animals after SIV infection controlled the virus poorly—the HVL animals. At the same time, the NK cells from a large group of uninfected animals presented with relatively uniform levels of NK cell cytolytic activity, suggesting that the higher pre-infection frequencies in the LVL cohort is not likely to be associated with a lower lytic function per unit cell. This is puzzling, as it was shown in the SIV model of CNS infection, that higher pre-infection NK cell activity actually correlated with less CNS damage and better prognosis (30). However, this can be possibly explained by

the fact, that this study used a highly neurotropic SIV isolate which targets the CNS compartment with differential replication dynamics and may not be a total reflection of the effect of the frequency of the NK cell population.

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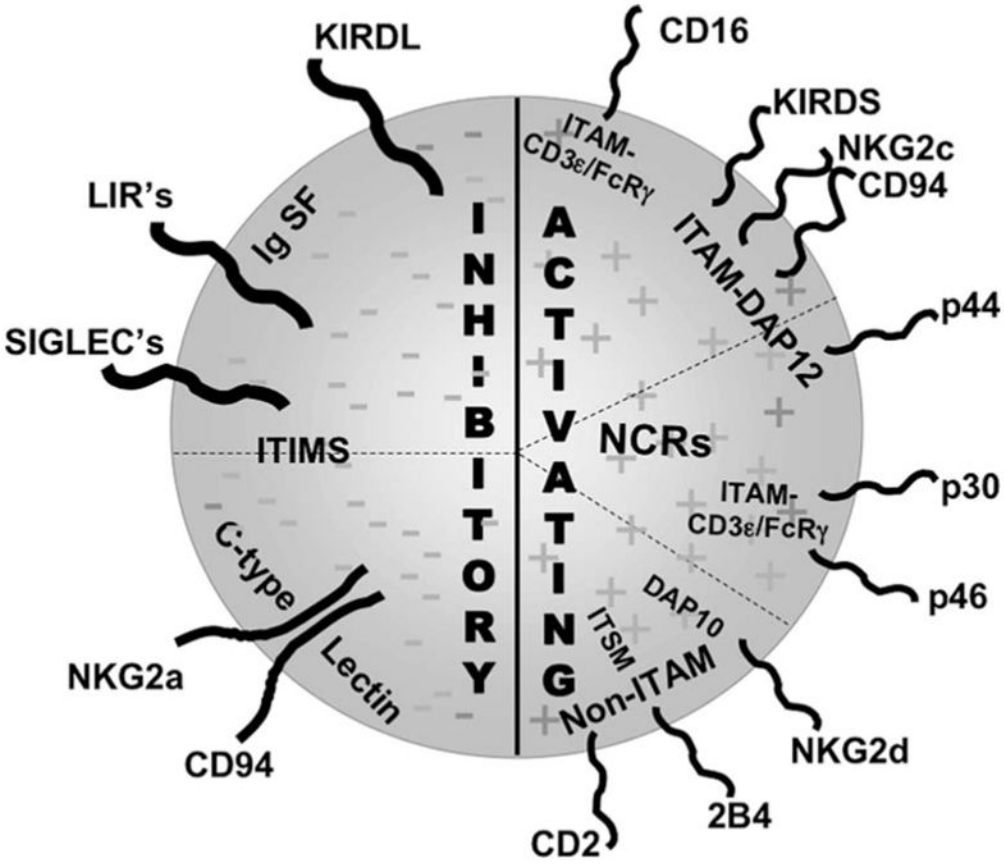


FIGURE 1.

NK cell surface receptors. NK cells express a variety of cell surface markers which can elicit negative or positive signals. The negative signal generating/inhibitory molecules include 1) the Ig superfamily molecules such as the killer cell Ig-like receptors with long cytoplasmic tails (KIRxDL), lymphocyte inhibitory receptors (LIRs) and sialic acid binding Ig-like lectins (SIGLECs), and 2) the C-type lectin receptors, which include NKG2a/CD94 heterodimer. The positive signal generating/activating receptors include 1) the ITAM-bearing molecules CD16, p30, and p46 (ITAM associated with FcRγ and the CD3ε); 2) ITAM bearing molecules NKG2c/CD94 heterodimer, KIRDS, and p44 (ITAM-bearing DAP12); and 3) the non-ITAM bearing receptors CD2, NKG2D (associated with DAP10), and 2B4 (associated with SAP polypeptide). NCR, natural cytotoxicity receptor.

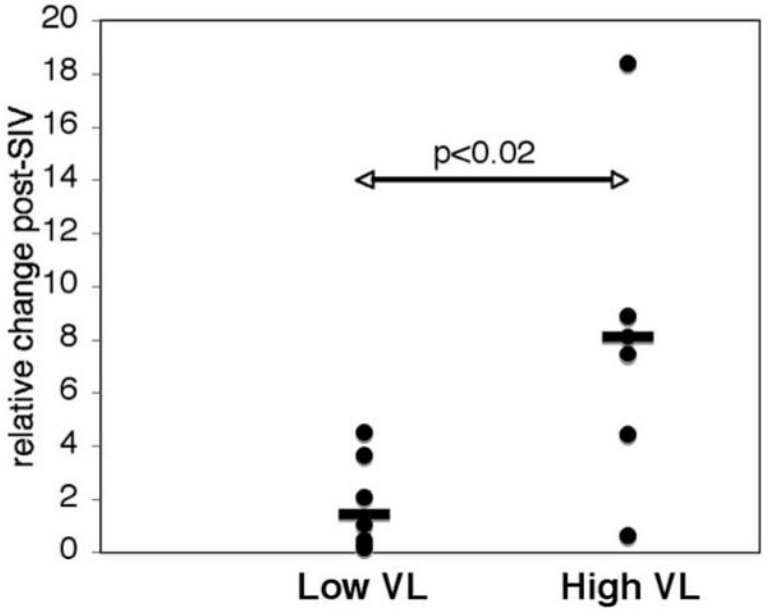


FIGURE 2.

KIR3DL expression in RMs pre- and post-SIV infection. Real-time PCR analysis for the levels of expression of KIR3DL cDNA in purified NK cells from 6 RM each from LVL and HVL cohorts. In each, animal quantitation was performed in cells pre- and post-SIV infection and results are expressed as fold change post-SIV vs pre-SIV.

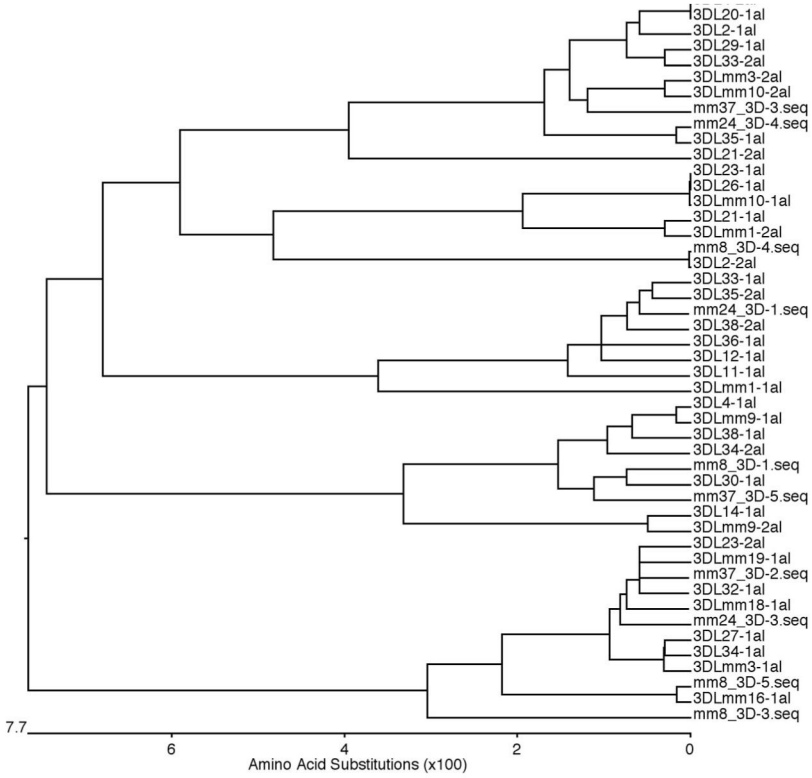


FIGURE 3.

Phylogenetic identification of KIR3DL alleles in RMs. Full-length sequences of KIR3DL from each animal were assigned into groups, based on the sequence similarity (>98%) and consensus sequences were generated representing “primary” individual alleles. These primary alleles were then compared between the animals based on the phylogenetic relationship (>98% similarity) and 14 alleles were defined within the population. The tree shown reflects phylogenetic relationship between the alleles formed by inidividual primary alleles (e.g., mm1–2al) and several individual sequences (xxxx.seq).

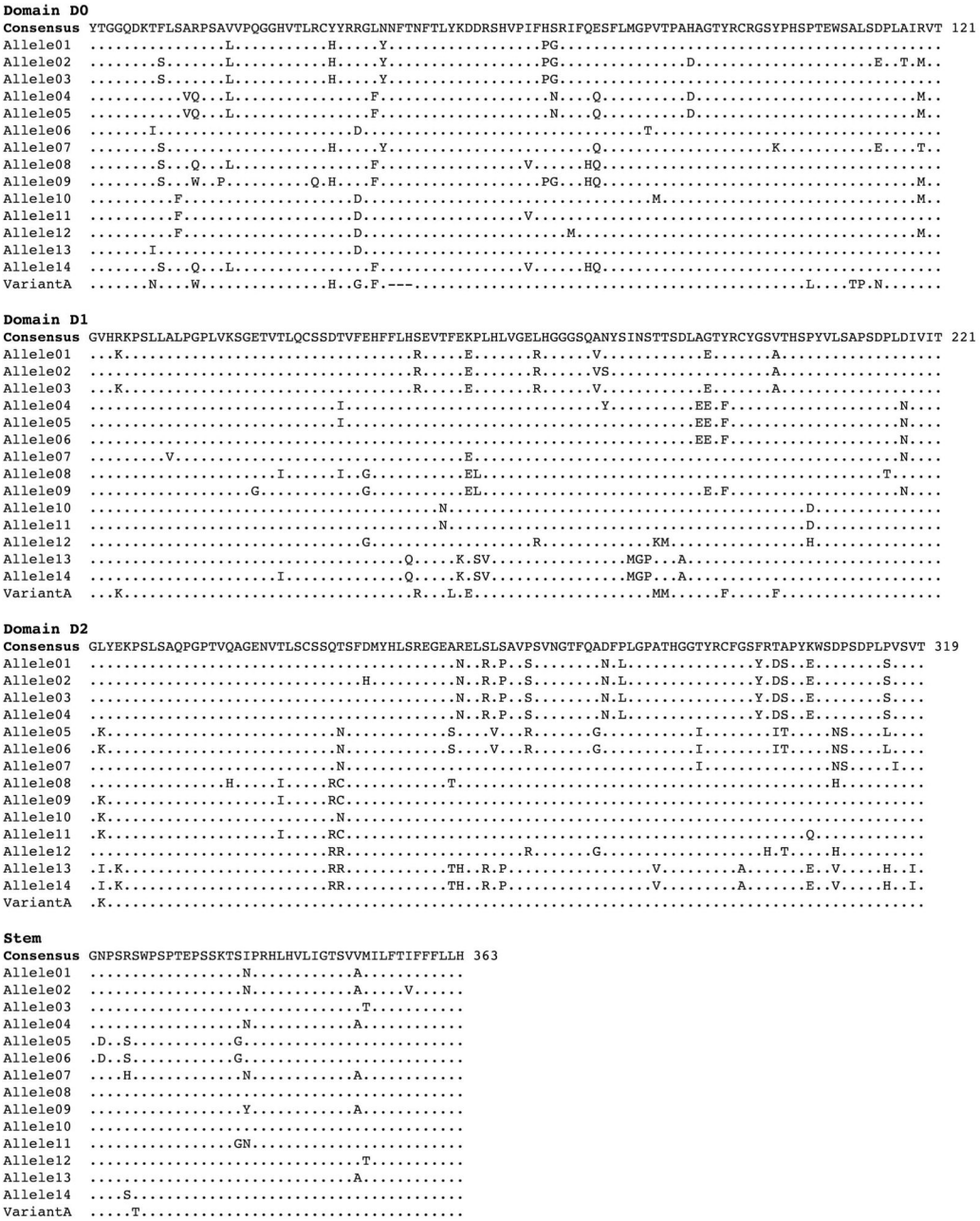
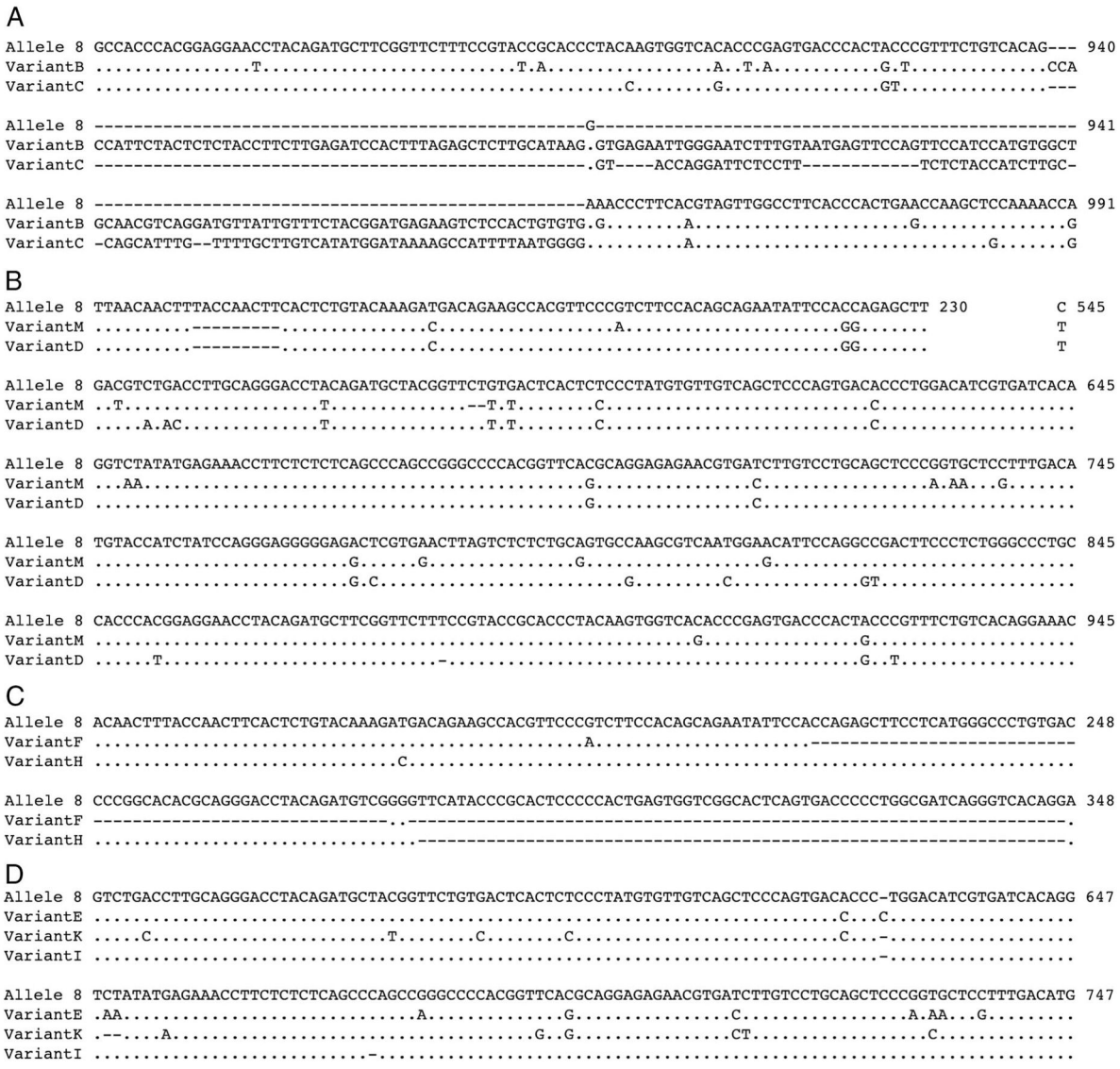


FIGURE 4.

Sequences of KIR3DL alleles in RMs. Alignment of protein sequences of 14 KIR3DL alleles (allele 1–14; GenBank accession numbers FJ562108 through FJ562121) and newly identified variant A. The three extracellular domains (D0, D1, and D2) and stem sequences are shown.



E

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Allele 8 CCCGGCACACGCAGGGACCTACAGATGTCGGGGTTCATACCCGCACTCCCCCACTGAGTGGTCGGCACTCAGTGACCCCCTGGCGATCAGGGTCACAGGA 348
VariantJ .................................A.................A.....................C.................T..........
VariantG .................................C.................................................G..............................
VariantL ..........................................................................................T..........
VariantN ..A.......A.........................C.....................................A.............T.........-

Allele 8 GTCCACAGAAAACCTTCCCTCCTGGCCCTCCCAGGTCCCCTGGTGAAATCAGGAGAGACGGTCATCCTACAATGTTCGTCAGATATCGTGTTTGGGCACT 448
VariantJ ..................................................................C.............C.......C..............
VariantG ..................................................................C.............C................A.....
VariantL ..................................................................C..................................
VariantN ----------------------------------------------------------------------------------------------------

Allele 8 TCTTTCTGCACAGTGAGGTGACCTTTGAGGAGCTCTTGCACCTTGTTGGAGAGCTCCATGGTGGGGGTTCCCAGGCCAACTACTCCATCAATTCCACGAC 548
VariantJ .................A..----------------------------------------------------------------------------
VariantG ..................----------------------------------------------------------------------------------
VariantL ..........................................................................A.......................
VariantN ----------------------------------------------------------------------------------------------------

Allele 8 GTCTGACCTTGCAGGGACCTACAGATGCTACGGTTCTGTGACTCACTCTCCCTATGTGTTGTCAGCTCCCAGTGACACCCTGGACATCGTGATCACAGGT 648
VariantJ -.............................T........C........C.A.........................C.......................
VariantG ---------------.........................C........CGA.........................C......................-
VariantL ..............A.....T..................C........C...........................C....AA..............--
VariantN -------------.--.......................C........C...G.....................................................

Allele 8 CTATATGAGAAACCTTCTCTCTCAGCCCAGCCGGGCCCCACGGTTCACGCAGGAGAGAACGTGATCTTGTCCTGCAGCTCCCGGTGCTCCTTTGACATGT 748
VariantJ ...............................A..............G.................C...................C...............
VariantG ----------------------------------------------------------------------------------------------------
VariantL ----------------------------------------------------------------------------------------------------
VariantN .............................................G.................C.................A.AA...G.C........

Allele 8 ACCATCTATCCAGGGAGGGGGAGACTCGTGAACTTAGTCTCTCTGCAGTGCCAAGCGTCAATGGAACATTCCAGGCCGACTTCCCTCTGGGCCCTGCCAC 848
VariantJ .......................G.C.........................G.........C.............G.....................
VariantG --------------.........G.......................G.....................................................
VariantL ----------------------------------------------.........A.....................................
VariantN .......................G.C....G...................A.........CA.....................................

Allele 8 CCACGGAGGAACCTACAGATGCTTCGGTTCTTTCCGTACCGCACCCTACAAGTGGTCACACCCGAGTGACCCACTACCCGTTTCTGTCACAGGAAACCCT 948
VariantJ ......................C............A....A.................................G.....................
VariantG ..................................................................G.............G.............A.........
VariantL .............................................G.........G.................G.....................
VariantN .............................................C........G.................GT.....................

Allele 8 TCACGTAGTTGGCCTTCACCCACTGAACCAAGCTCCAAAACCAGTATCCCC-AGACACCTGCATGTTCTGATTGGGACCTCAGTGGTCATGATCCTCTTC 1047
VariantJ ...G.........................A.........TG.....A..-..........C.A..G.....A..TA...G....C..CC...A.....
VariantG ...........................................................-.....................................................
VariantL ....................................G.......C.....................................C..........
VariantN ...A..............................G.......G.....A..-..........C.A..G.....A..TA...G....C..CC...A.....

Allele 8 ACCATC---TTCTTCTTTCTCCTGCATCGCTG
VariantJ ......CTC.....................
VariantG ......---.....................
VariantL ......---.....................
VariantN ......CTC.....................
```

FIGURE 5.

Sequences of new KIR3DL variants. Nucleotide sequences of newly identified variants B–N are aligned with the sequence of allele 8: *A*, variants B and C; *B*, variants D and M; *C*, variants F and H; *D*, variants E, K, and I; and *E*, variants J, G, L, and N. Partial alignments of each set of variants are shown, depicting the part of the sequence with major variation against the allele 8. Sequence numbering reflects AF334616.

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RM cons  YTGGQDKTFLSARPSAVVPQGGHVTLRCYYRRGLNNFTNFTLYKDDRSHVPIFHSRIFQESFLMGPVTPAHAGTYRCRGSYPHSPTEWSALSDPLAIRVT 121
Allele 8 ........S...Q...L.................F.................V......HQ.....................................
Allele13 .......I.......................D.....................................................................
Allele14 ........S...Q...L.................F.................V......HQ.....................................
HuKIR3DL HM.....P....W......R........H..HRF.---..M...E..I.I....G.......N.S...T....N.T....H.....G...P.N.VV.M..

RM cons  GVHRKPSLLALPGPLVKSGETVTLQCSSDTVFEHFFLHSEVTFEKPLHLVGELHGGGSQANYSINSTTSDLAGTYRCYGSVTHSPYVLSAPSDPLDIVIT 221
Allele 8 ..........F..................I..G...........EL.............................................T......
Allele13 .....................................Q.....K.SV.................MGP...A.....................................
Allele14 ......................I..............Q.....K.SV.................MGP...A.....................................
HuKIR3DL .N........H.........R.I...W..IM.......K.GISKD.SR...QI.D.V.K..F..GPMMLA.............T..Q...........V.

RM cons  GLYEKPSLSAQPGPTVQAGENVTLSCSSQTSFDMYHLSREGEARELSLSAVPSVNGTFQADFPLGPATHGGTYRCFGSFRTAPYKWSDPSDPLPVSVTGN 321
Allele 8 ................H.....I.....RC.............T.............................................H............
Allele13 .I.K.........................RR.............TH..R.P.................V.........A.......E..V.....H..I...
Allele14 .I.K.........................RR.............TH..R.P.................V.........A.......E..V.....H..I...
HuKIR3DL .P............K.....S.......RS.Y.........G.H.RR.P..RK..R.......................HS..E........L......

RM cons  PSRSWPSPTEPSSKTSIPRHLHVLIGTSVVMILFT-IFFFLLHRWCSNKKNAAVKDQEPAGDRTVNREDSEEQDPEEVTYAQLDHCVLTQGKTTRPSQRR 420
Allele 8 ..................................-.....................................................................
Allele13 ............................A.....-.....................................L.........R......I.H...KP
Allele14 ..S.............................-..................M..............PD....Q.........R......I.....KP
HuKIR3DL ..S............SGN.....I.......I...ILLL.....L..........M......N..A.S...D................F..R.I......P

RM cons  KRPPTDTSVYIELPNAESRSKVVSCP
Allele 8 ..........................
Allele13 .T........T......P.....FY.
Allele14 .T........T......P.....FY.
HuKIR3DL .T.....IL.T.....KP........
```

FIGURE 6.

Sequences of the most prevalent KIR3DL alleles in RMs. Alignment of protein sequences of RM KIR3DL alleles 13, 14, and 8, together with comparison to the sequence of human KIR3DL (Hu KIR3DL; NM_013289) and RM consensus sequence (RM consensus). The three extracellular domains, stem, and cytoplasmic tail sequences are shown.

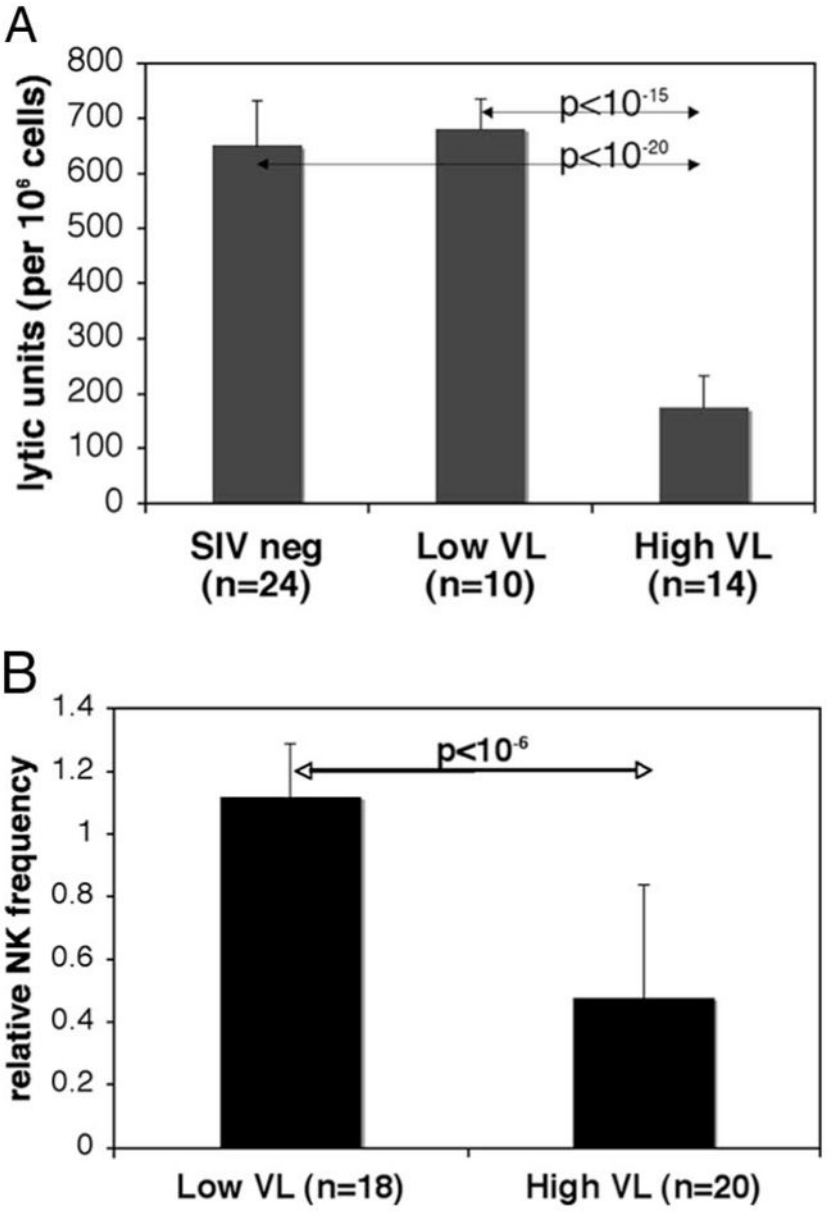

FIGURE 7.

NK cell function in RMs pre- and post-SIV infection. *A*, Purified NK cells from SIV naive (SIV uninfected) and majority of SIV-infected animals from both HVL and LVL cohorts were analyzed for their lytic activity. Means of each group are displayed. *B*, PBMC from RM pre-and post-SIV infection were analyzed in two cohorts—high viral load (HVL) and low viral load (LVL)—for frequency of NK cells ($CD3^{-}CD8^{+}$). Relative change in NK cell frequency for each animal is first calculated as frequency post-SIV/frequency pre-SIV and means of these relative individual changes are displayed for each cohort.

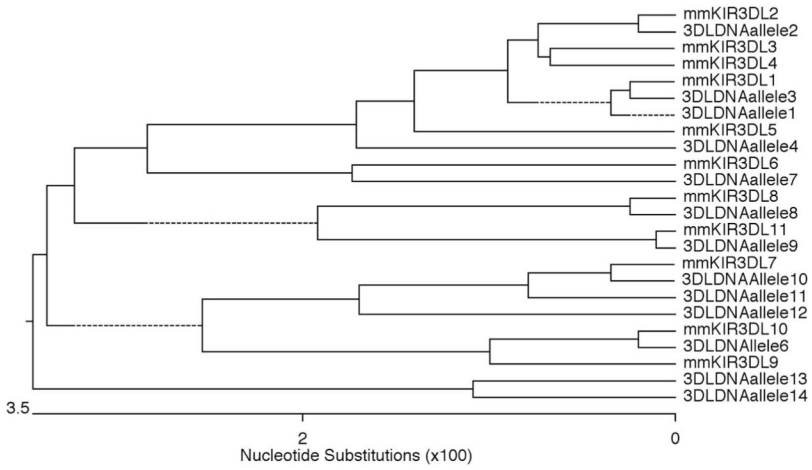

FIGURE 8.

Phylogenetic relationship between previously defined KIR3DL variants and newly defined alleles. The phylogenetic distance tree depicts the relationship between the 11 previously described full-length KIR3DL variants (mm KIR3DL 1–11) (31) and 14 newly defined alleles (3DLDNAAllele 1–14).

Table I

KIR3DL-related clones obtained from RM[a]

Macaque ID	Clone 1	Clone 2	Clone 3	Clone 4	Clone 5
mm01	3DL	3DL	3DL	3DL	3DL
mm02	3DL	3DL	sv2	sv2	sv2
mm03	3DL	3DL	3DL	3DL	3DL
mm04	3DL	3DL	3DL	3DL	sv5
mm05	sv2	sv2	sv2	sv2	sv2
mm06	sv2	sv2	sv2	sv2	sv2
mm07	sv2	sv2	sv2	sv2	sv2
mm08	3DL	3DL	3DL	3DL	var E
mm09	3DL	3DL	3DL	var A	var A
mm10	3DL	3DL	3DL	sv2	var B
mm11	3DL	3DL	3DL	3DL	3DL
mm12	3DL	3DL	3DL	3DL	3DL
mm13	sv2	sv2	sv2	sv2	sv2
mm14	3DL	sv2	sv2	sv2	sv2
mm15	sv2	sv2	sv2	sv2	sv2
mm16	3DL	3DL	sv2	sv2	sv2
mm17	sv2	sv2	sv2	sv2	sv2
mm18	3DL	3DL	sv2	sv2	sv2
mm19	3DL	3DL	sv2	var C	var D
mm20	3DL	3DL	3DL	3DL	var A
mm21	3DL	3DL	3DL	3DL	sv7
mm22					
mm23	3DL	3DL	3DL	3DL	var D
mm24	3DL	3DL	3DL	var A	var A
mm25					
mm26	3DL	3DL	sv5	var F	var F
mm27	3DL	3DL	3DL	var G	var G
mm28					

Macaque ID	Clone 1	Clone 2	Clone 3	Clone 4	Clone 5
mm29	3DL	3DL	3DL	3DL	3DL
mm30	3DL	3DL	3DL	3DL	3DL
mm31					
mm32	3DL	3DL	3DL	var A	var A
mm33	3DL	3DL	3DL	sv2	sv2
mm34	3DL	3DL	3DL	3DL	var K
mm35	3DL	3DL	3DL	var H	var I
mm36	3DL	3DL	3DL	3DL	var A
mm37	3DL	3DL	3DL	var L	var N
mm38	3DL	3DL	3DL	var M	var M

[a]3DL, full length clones; sv2, sv5, sv7, splice variants as described by Hershberger et al. (31); var A–N, new variants.

Table II

KIR3DL allele distribution among RMs

Macaque ID	Alleles (full length)	Splice Variant 2[a]	New Variant	MHC Class I (Mamu)[b]
mm01	6, 9			
mm02	1, 7	8		
mm03	2, 13			
mm04	1, 10			
mm05		8		
mm06		8		
mm07		8		B.01
mm08	7, 11, 14		E	B.01
mm09	10, 12		A	B.01
mm10	2, 5	5	B	
mm11	8			
mm12	8			
mm13		8		A.01, B.01, B.17
mm14	12	8		A.01
mm15		8		A.01
mm16	14			
mm17		8		
mm18	13	8		
mm19	13	1	C, D	A.01
mm20	1		A	A.01
mm21	4, 6			
mm22				
mm23	5, 13		D	A.01
mm24	3, 8, 13		A	B.01
mm25				
mm26	5		F	
mm27	13		G	
mm28				
mm29	1			
mm30	11			
mm31				
mm32	13		A	A.01
mm33	1, 8	8		
mm34	10, 13		K	A.01
mm35	3, 8		H, I	
mm36	8		A	B.08
mm37	13		L, N	B.17
mm38	8, 10		M	A.01

[a] Allele with the highest sequence homology to the sv2 sequence obtained.

[b] Animals typed for Mamu A.01, B.01, B.08, and B.17; only positive results are listed.

Table III

Frequency of KIR3DL1 alleles in rhesus macaques[a]

	ALL (*n* = 28)	HVL (*n* = 15)	LVL (*n* = 13)
Allele 1	0.18	0.13	0.23
Allele 2	0.07	0.13	0
Allele 3	0.07	0.07	0.08
Allele 4	0.04	0	0.08
Allele 5	0.11	0.2	0
Allele 6	0.07	0.07	0.08
Allele 7	0.07	0.07	0.08
Allele 8	0.25	0.13	0.38
Allele 9	0.04	0.07	0
Allele 10	0.14	0.13	0.15
Allele 11	0.07	0.07	0.08
Allele 12	0.07	0.07	0.08
Allele 13	0.32	0.47	0.15
Allele 14	0.07	0.13	0
Allele 13/14	0.39	0.6	0.15
Variant A	0.18	0.13	0.23

[a]ALL, all animals.

Table IV

Frequency of 159 H-Q SNP among rhesus macaques by TaqMan[a]

Genotype	ALL (*n* = 38)	HVL (*n* = 20)	LVL (*n* = 18)
H/H	0.30	0.16	0.58
H/Q	0.70	0.84	0.42

[a]ALL, all animals.