Published in final edited form as:

Immunogenetics. 2015 October ; 67(10): 625–628. doi:10.1007/s00251-015-0863-0.

Chimpanzee susceptibility to hepatitis C virus infection correlates with presence of *Pt-KIR3DS2* and *Pt-KIR2DL9*: paired activating and inhibitory natural killer cell receptors

Laurent Abi-Rached^{1,2}, Lisbeth A. Guethlein¹, Paul J. Norman¹, and Peter Parham¹

Lisbeth A. Guethlein: lisbeth.guethlein@stanford.edu

¹Departments of Structural Biology and Microbiology and Immunology, Stanford University, Fairchild D-159, 299 Campus Drive West, Stanford, CA 94305, USA

Abstract

Infection of humans and chimpanzees with Hepatitis C virus (HCV) results in either the resolution of the acute infection or its progression to a persistent infection associated with chronic liver disease. In cohorts of human patients, resolution of HCV infection has been associated with homozygosity for both C1⁺HLA-C and its cognate inhibitory receptor, KIR2DL3. Compared here are the killer cell immunoglobulin-like receptors (KIR) and major histocompatibility complex (MHC) class I factors of chimpanzees who resolve, or resist, HCV infection with those chimpanzees who progress to chronic infection. Analysis of *Pt-KIR* gene content diversity associated two of the 12 *Pt-KIR* with clinical outcome. Activating *Pt-KIR3DS2* and inhibitory *Pt-KIR2DL9* are strong receptors specific for the C2 epitope. They are encoded by neighboring genes within the *Pt-KIR* locus that are in strong linkage disequilibrium. HCV-infected chimpanzees with *KIR* genotypes containing *Pt-KIR3DS2* and *KIR2DL9* are significantly more likely to progress to chronic infection than infected chimpanzees lacking the genes ($p=0.0123$ and $p=0.0045$, respectively), whereas human HLA-B allotypes having the C1 epitope are unusual, such allotypes comprise about one quarter of the chimpanzee *Patr-B* allotypes. Homozygous *C1⁺Patr-B* are enriched in chimpanzees with chronic HCV infection, and the compound genotype of homozygous *C1⁺Patr-B* combined with either *Pt-KIR3DS2* or *Pt-KIR2DL9* is more strongly associated with disease progression than either factor alone ($p=0.0031$ and $p=0.0013$, respectively). Thus, despite similarities suggesting a common basis in disease resistance, there are substantial differences in the KIR and MHC class I correlations observed for HCV-infected humans and chimpanzees, consistent with the divergence of their KIR and MHC class I systems.

Keywords

MHC; KIR; Hepatitis C virus; Chronic infection; Chimpanzee

Natural killer (NK) cells are vital lymphocytes that contribute to innate immunity and adaptive immunity and also to reproduction during formation of the placenta (Parham and

Correspondence to: Lisbeth A. Guethlein, lisbeth.guethlein@stanford.edu.

²Present address: Equipe ATIP, URMITE UM 63 CNRS 7278 IRD 198 Inserm U1095, IHU Méditerranée Infection, Aix-Marseille Université, Marseille, France

Moffett 2013). In the immune system, NK cells are prominent in providing defense against viral infections (Lanier 2008). The education of NK cells, which teaches them to identify and kill virus-infected cells, is dependent upon NK cell receptors that recognize polymorphic determinants of MHC class I molecules (Watzl 2014). In humans, the diverse family of killer cell immunoglobulin-like receptors (KIR) provides NK cell receptors that recognize polymorphic determinants of HLA-A, -B, and -C molecules (Parham and Moffett 2013). Of particular importance are the inhibitory and activating KIR2D that recognize the C1 and C2 epitopes of HLA-C. These epitopes are determined by dimorphism at position 80 in the α_1 domain, where asparagine and lysine specify the C1 and C2 epitopes, respectively (Colonna et al. 1993). The *KIR2DL2/3* gene that encodes inhibitory C1 receptors is polymorphic and comprises two distinct lineages of alleles called *KIR2DL2* and *KIR2DL3*. In an epidemiological study of hepatitis C virus (HCV) infection, Khakoo et al. showed that resolution of acute HCV infection was associated with homozygosity for KIR2DL3 and C1-bearing HLA-C in cohorts of Europeans and African-Americans (Khakoo et al. 2004). Corroborating this finding is the observed association between C2 epitope homozygosity and the failure to respond to HCV treatment (Suppiah et al. 2011).

The chimpanzee is the only good animal model for human HCV infection. In both humans and chimpanzees, some infected individuals are able to resolve the acute infection, while others develop a chronic infection that can lead to severe liver disease. A majority of humans infected with HCV (~75–85 %) become chronically infected (<http://www.cdc.gov>), whereas it is a minority (~30 %) of chimpanzees that develop chronic infections (Bettauer 2010). The chimpanzee is also the species for which the genetics and immunogenetics are most like their human counterparts (Adams and Parham 2001; Prado-Martinez et al. 2013). The chimpanzee (*Pan troglodytes*) has strict Patr-A, -B, and -C orthologs of human HLA-A, -B, and -C, and has both the C1 and C2 epitopes that are recognized by activating and inhibitory KIR (Adams and Parham 2001; Moesta et al. 2009). One difference between the two species is that a significant proportion of the allotypes of Patr-B, the chimpanzee ortholog of HLA-B, has the C1 epitope and is recognized by C1-specific KIR. Only two HLA-B allotypes have the C1 epitope: HLA-B*73:01 is rare and HLA-B*46 is restricted to Southeastern Asian populations (Abi-Rached et al. 2010). In contrast to MHC class I, the human and chimpanzee KIR (Pt-KIR) recognizing MHC-C are not strict orthologs but form part of the same lineage of KIR, called lineage III (Khakoo et al. 2000). Distinguishing the chimpanzee *KIR* locus is a greater diversity of C1-specific and C2-specific receptors; distinguishing the human *KIR* locus is the two distinctive *A* and *B* forms of *KIR* haplotype that appear subject to different selective pressure (Abi-Rached et al. 2010).

In defining the structure and variability of the chimpanzee *KIR* gene family, we studied a panel of 39 chimpanzees comprising 35 western chimpanzees (*Pan troglodytes verus*), two eastern chimpanzees (*Pan troglodytes schweinfurthii*), one central chimpanzee (*Pan troglodytes troglodytes*), and one individual for whom the subspecies is unknown (Abi-Rached et al. 2010). That 24 of the individuals in this panel had been subjects for experimental HCV infection and vaccination studies (Cooper et al. 1999) enabled us to explore the influence of KIR and MHC class I type on the outcome of HCV infection.

Major features of *KIR* diversity are that haplotypes differ in both number and type of genes they contain. This is clearly seen in Fig. 1, which compares the presence and absence of 12 *Pt-KIR* genes in the cohort of 24 chimpanzees. These genes include eight that encode MHC-C receptors: three C1 receptors (activating *Pt-KIR3DS6* and inhibitory *Pt-KIR2DL6* and *Pt-KIR2DL8*) and five C2 receptors (activating *Pt-KIR3DS2*, and inhibitory *Pt-KIR2DL9*, *Pt-KIR3DL5*, *Pt-KIR3DL4*, and *Pt-KIR2DL7*). The other four genes encode *Pt-KIR3DL3*, *Pt-KIR2DL4*, and *Pt-KIR2DL5*, for which ligands are not established, and *Pt-KD3L1/2* that recognizes MHC-A and MHC-B (Khakoo et al. 2000). Two divergent “a” and “b” allotypes of *Pt-KIR3DL1/2* (formerly *Pt-KIR3DL1/2* and *Pt-KIR3DL3* in (Khakoo et al. 2000)) are distinguished in Fig. 1.

The cohort of 24 chimpanzees divides into a first group of 11 chimpanzees that resisted HCV infection (Fig. 1, upper part) and a second group of chimpanzees that failed to resist HCV and developed chronic infection (Fig. 1, lower part). The first group consists of two individuals (LouLou B and Todd) who were not vaccinated and resolved acute HCV infection, four individuals who were vaccinated and became protected against subsequent challenge with the virus, and five vaccinated individuals who became infected on subsequent challenge with HCV and then resolved the infection. The second group, of 13 chimpanzees, developed chronic infection on viral challenge, seven of whom were vaccinated and six who were not (Cooper et al. 1999). As is clearly seen in Fig. 1, of the 12 chimpanzee *KIR* genes, *Pt-KIR3DS2* and *Pt-KIR2DL9* are more highly represented in the group with chronic disease (10 of 13 individuals) than in the group who resisted HCV (two of 11 individuals). Absence of these two *Pt-KIR* genes correlates with resistance and resolution of HCV infection, with an odds ratio (OR) of 22.5 ($p=0.0045$) for *Pt-KIR2DL9* and 15.0 ($p=0.0123$) for *Pt-KIR3DS2* (Fig. 2). None of the other *KIR* varies significantly between the groups of resistant and susceptible chimpanzees, as is exemplified by the OR of 0.8 for *Pt-KIR3DL5* and the OR of 0.5 for *Pt-KIR3DS6*.

The order of the *Pt-KIR* genes in Fig. 1 reflects their order in the chimpanzee *Pt-KIR* locus (Abi-Rached et al. 2010). Thus, *Pt-KIR3DS2* and *Pt-KIR2DL9* are seen to be neighboring genes (Fig. 1). Moreover, they are only 2 kbp apart because of the characteristically short and homologous intergenic sequences that separate *KIR* genes (Wilson et al. 2000). As a consequence there is strong linkage disequilibrium (LD) between *Pt-KIR3DS2* and *Pt-KIR2DL9*, as seen from only two of the 24 chimpanzees having one of these genes and not the other, as well as comparison of chimpanzee *KIR* haplotypes (Abi-Rached et al. 2010). Selection could also be having a role in maintaining the linkage between *Pt-KIR3DS2* and *Pt-KIR2DL9*, which encode strong activating and inhibitory C2 receptors, respectively.

We compared the groups of resistant and susceptible chimpanzees for differences in the distribution of the C1 and C2 epitopes of MHC-C, but no significant differences were observed. However, an interesting trend was observed in the segregation of alleles encoding $C1^{+}Patr-B$ allotypes. Indeed, that three individuals of the susceptible group are homozygous for $C1^{+}Patr-B$, compared to none of the resistant group suggested that homozygosity for C1-bearing $Patr-B$ could be a risk factor for chronic infection and that its absence might favor resolution of infection. Consistent with this hypothesis, the compound genotype, defined by absence of both *Pt-KIR2DL9* and homozygous $C1^{+}Patr-B$, was more strongly

correlated with resolution of HCV infection (OR of 33.3; $p=0.0013$) than either factor alone. Similarly, the absence of *Pt-KIR3DS2* combined with absence of homozygous *C1⁺Patr-B* favored resolution of infection (OR of 24.8; $p=0.0031$).

The resolution of human HCV infection was correlated with homozygosity for C1-specific KIR2DL3 and for C1⁺HLA-C (Khakoo et al. 2004). In that analysis, heterozygosity for C1⁺HLA-C, which is due to the *C1⁺HLA-C/C2⁺HLA-C* genotype, provided little improvement in outcome, suggesting that absence of C2 could be an important factor for resolving HCV infection. Supporting this hypothesis is the association of C2 homozygosity with failure to respond to treatment for HCV (Suppiah et al. 2011). In a C1⁺HLA-C homozygote, but not a heterozygote, the strong inhibitory C2 receptor KIR2DL1 is rendered nonfunctional. Although accomplished by a different mechanism, a similar trend is seen in the chimpanzee model. Elimination of the strong C2 receptors, *Pt-KIR3DS2* and *Pt-KIR2DL9*, is associated with resistance to HCV infection. A possible consequence of the absence of these receptors is that the C2 ligand is rendered less functional, and NK cell education becomes more targeted to the C1 epitope and its interactions with C1-specific *Pt-KIR*. In this circumstance the gene dose of the C1 epitope could become a critical factor and explain our observation that C1⁺Patr-B homozygosity is detrimental for resolving HCV infection. Among the chimpanzees that resolved infection are ones with 0–3 copies of C1 but none with four copies. In contrast, two of the three chimpanzees, with chronic infection and who are C1⁺Patr-B homozygous, encode four copies of the C1 epitope. NK cell education is sensitive to the abundance of the KIR ligand on the NK cell surface (Orr and Lanier 2010), and the expression of MHC-B is considerably greater than that of MHC-C (Apps et al. 2015). Thus, genotypes with two genes encoding C1⁺Patr-B could reduce the effectiveness of C1-mediated NK cell education.

Acknowledgements

Chimpanzee blood samples were provided by the Yerkes Regional Primate Center. This study was supported by the National Institutes of Health grants AI24258 and AI31168 to PP and the Yerkes Base Grant ORIP/OD P51OD011132.

References

- Abi-Rached L, Moesta AK, Rajalingam R, Guethlein LA, Parham P. Human-specific evolution and adaptation led to major qualitative differences in the variable receptors of human and chimpanzee natural killer cells. *PLoS Genet.* 2010; 6:e1001192. [PubMed: 21079681]
- Adams EJ, Parham P. Species-specific evolution of MHC class I genes in the higher primates. *Immunol Rev.* 2001; 183:41–64. [PubMed: 11782246]
- Apps R, Meng Z, Del Prete GQ, Lifson JD, Zhou M, Carrington M. Relative expression levels of the HLA class-I proteins in normal and HIV-infected cells. *J Immunol.* 2015; 194:3594–3600. [PubMed: 25754738]
- Bettauer RH. Chimpanzees in hepatitis C virus research: 1998–2007. *J Med Primatol.* 2010; 39:9–23. [PubMed: 19900169]
- Choo QL, Kuo G, Ralston R, Weiner A, Chien D, Van Nest G, Han J, Berger K, Thudium K, Kuo C, et al. Vaccination of chimpanzees against infection by the hepatitis C virus. *Proc Natl Acad Sci U S A.* 1994; 91:1294–1298. [PubMed: 7509068]
- Colonna M, Brooks EG, Falco M, Ferrara GB, Strominger JL. Generation of allospecific natural killer cells by stimulation across a polymorphism of HLA-C. *Science.* 1993; 260:1121–1124. [PubMed: 8493555]

- Cooper S, Erickson AL, Adams EJ, Kansopon J, Weiner AJ, Chien DY, Houghton M, Parham P, Walker CM. Analysis of a successful immune response against hepatitis C virus. *Immunity*. 1999; 10:439–449. [PubMed: 10229187]
- Khakoo SI, Rajalingam R, Shum BP, Weidenbach K, Flodin L, Muir DG, Canavez F, Cooper SL, Valiante NM, Lanier LL, Parham P. Rapid evolution of NK cell receptor systems demonstrated by comparison of chimpanzees and humans. *Immunity*. 2000; 12:687–698. [PubMed: 10894168]
- Khakoo SI, Thio CL, Martin MP, Brooks CR, Gao X, Astemborski J, Cheng J, Goedert JJ, Vlahov D, Hilgartner M, Cox S, Little AM, Alexander GJ, Cramp ME, O'Brien SJ, Rosenberg WM, Thomas DL, Carrington M. HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. *Science*. 2004; 305:872–874. [PubMed: 15297676]
- Lanier LL. Evolutionary struggles between NK cells and viruses. *Nat Rev Immunol*. 2008; 8:259–268. [PubMed: 18340344]
- Moesta AK, Abi-Rached L, Norman PJ, Parham P. Chimpanzees use more varied receptors and ligands than humans for inhibitory killer cell Ig-like receptor recognition of the MHC-C1 and MHC-C2 epitopes. *J Immunol*. 2009; 182:3628–3637. [PubMed: 19265141]
- Orr MT, Lanier LL. Natural killer cell education and tolerance. *Cell*. 2010; 142:847–856. [PubMed: 20850008]
- Parham P, Moffett A. Variable NK cell receptors and their MHC class I ligands in immunity, reproduction and human evolution. *Nat Rev Immunol*. 2013; 13:133–144. [PubMed: 23334245]
- Prado-Martinez J, Sudmant PH, Kidd JM, Li H, Kelley JL, Lorente-Galdos B, Veeramah KR, Woerner AE, O'Connor TD, Santpere G, Cagan A, Theunert C, Casals F, Laayouni H, Munch K, Hobolth A, Halager AE, Malig M, Hernandez-Rodriguez J, Hernando-Herraez I, Prufer K, Pybus M, Johnstone L, Lachmann M, Alkan C, Twigg D, Petit N, Baker C, Hormozdiari F, Fernandez-Callejo M, Dabad M, Wilson ML, Stevison L, Camprubi C, Carvalho T, Ruiz-Herrera A, Vives L, Mele M, Abello T, Kondova I, Bontrop RE, Pusey A, Lankester F, Kiyang JA, Bergl RA, Lonsdorf E, Myers S, Ventura M, Gagneux P, Comas D, Siegmund H, Blanc J, Agueda-Calpena L, Gut M, Fulton L, Tishkoff SA, Mullikin JC, Wilson RK, Gut IG, Gonder MK, Ryder OA, Hahn BH, Navarro A, Akey JM, Bertranpetit J, Reich D, Mailund T, Schierup MH, Hvilsom C, Andres AM, Wall JD, Bustamante CD, Hammer MF, Eichler EE, Marques-Bonet T. Great ape genetic diversity and population history. *Nature*. 2013; 499:471–475. [PubMed: 23823723]
- Suppiah V, Gaudieri S, Armstrong NJ, O'Connor KS, Berg T, Weltman M, Lorena Abate M, Spengler U, Bassendine M, Dore GJ. IL28B, HLA-C, and KIR variants additively predict response to therapy in chronic hepatitis C virus infection in a European Cohort: a cross-sectional study. *PLoS Med*. 2011; 8:1243.
- Watzl C. How to trigger a killer: modulation of natural killer cell reactivity on many levels. *Adv Immunol*. 2014; 124:137–170. [PubMed: 25175775]
- Wilson MJ, Torkar M, Haude A, Milne S, Jones T, Sheer D, Beck S, Trowsdale J. Plasticity in the organization and sequences of human KIR/ILT gene families. *Proc Natl Acad Sci U S A*. 2000; 97:4778–4783. [PubMed: 10781084]

Chimpanzee	MHC				KIR													HCV infection outcome	Virus dose	Vaccine
	Patr-B		Patr-C		3DL3	3DS2	2DL9	3DS6	2DL6	2DL5	2DL8	3DL5	3DL4	2DS4	2DL7	2DL4	3DL1/2 a b			
LouLouB	C1		C1	C2														Resolved	10	Mock
Todd	C1		C1	C2															64	None
Eve			C1	C1															10	Yes
Jena	C1		C1	C1															10	Yes
MissEve			C2	C2															100	Yes
Kareem			C1	C2															10	Yes
Dali			C2	C2															10	Yes
FredAstaire			C2	C2															10	Yes
Paula			C2	C2														Protected	10	Yes
Tibi	C1		C1	C1															10	Yes
Chatter			C1	C1															10	Yes
CherylB			C2	C2														Chronic	10	Yes
Briggsie			C1	C2															10	Yes
Winnie	C1	C1	C2	C2															10	Yes
Hulk	C1		C1	C2															10	Yes
Ross	C1	C1	C1	C1															100	Yes
Blair	C1		C2	C2															1	None
Harry			C1	C1															10	Yes
Nemo			C1	C2															10	None
Buckwheat	C1		C1	C2															10	None
Mahoney	C1	C1	C1	C1															10	Yes
Mouse			C1	C2															10	None
Reggie			C1	C2															100	None
Termite			C2	C2															10	None

Fig. 1.

Absence of the C2-specific Pt-KIR2DL9 (inhibitory) and Pt-KIR3DS2 (activating) favors resolution of chimpanzee HCV infection. Shows *Pt-KIR* and *MHC C1/C2* content in a panel of 24 chimpanzees infected with HCV. C1 and C2 contents are listed on the left side of the panel and include C1 contributed by C1⁺ Patr-B (allotypes with valine 76 and asparagine 80). *Pt-KIR* gene content is shown in the central part of the panel: lineage III *Pt-KIR* with known specificities for MHC class I are colored either *blue* (C1 specific) or *pink* (C2 specific), and specificity is given below their names; names of activating KIR are in *green*. Virus doses (expressed in 50 % chimpanzee infectious doses, CID₅₀) used for postvaccination challenge, vaccination status, and HCV infection outcome are listed on the right side of the panel and have been published; for Paula (#W176), Tibi (#L559) and Chatter (#L357) (Choo et al. 1994) and for the remaining individuals (Cooper et al. 1999). The vaccination and challenge protocols have also been described previously (Choo et al. 1994; Cooper et al. 1999). The population and *KIR* typing methods are described in (Abi-Rached et al. 2010). *MHC-B* and *-C* content was previously completely characterized (two alleles per locus) for 13 of the 24 individuals for *Patr-C* and for 18 of the 24 individuals for *Patr-B* (Cooper et al. 1999). We investigated the *C1/C2* content at the *MHC-B* and *-C* loci for the remaining individuals by amplification and sequencing of exon 2, which encodes the $\alpha 1$ domain, the domain that contains the C1 or C2 epitope (PCR primers and amplification conditions are available on request). This study was approved by the Stanford University administrative panels on human subjects in medical research and laboratory animal care

Genetic Factor		Resolved N (%)	Persistent N (%)	OR	95% CI	P
KIR	2DL9	10 (90.9)	4 (30.8)	22.5	2.1 - 240.5	0.0045
	3DS2	9 (81.8)	3 (23.1)	15.0	2.0 - 111.2	0.0123
	3DL5	9 (81.8)	11 (84.6)	0.8	0.1 - 7.0	NS
	3DS6	8 (72.7)	11 (84.6)	0.5	0.1 - 3.6	NS
MHC	C C1	7 (63.6)	9 (69.2)	0.8	0.1 - 4.3	NS
	C C2	7 (63.6)	10 (76.9)	0.5	0.1 - 3.1	NS
	C C1/C1	4 (36.4)	3 (23.1)	1.9	0.3 - 11.3	NS
	C C2/C2	4 (36.4)	4 (30.8)	1.3	0.2 - 7.1	NS
	B C1	4 (36.4)	6 (46.2)	0.7	0.1 - 3.4	NS
	B C1/C1	11 (100.0)	10 (76.9)	NA	NA	NS
	C1 or C2 only	8 (72.7)	5 (38.5)	4.3	0.8 - 24.2	NS
B C1/C1 and 2DL9		10 (90.9)	3 (23.1)	33.3	2.9 - 377.5	0.0013
B C1/C1 and 3DS2		9 (81.8)	2 (15.4)	24.8	2.9 - 212.2	0.0031

Fig. 2.

Statistical analyses of the correlations between chimpanzee MHC class I, KIR, and resistance to HCV infection. Shown are *Pt-KIR 2DL9, 3DS2, 3DL5, 3DS6* and *MHC-C C1, C2, C1/C1, C2/C2, MHC-B C1, C1/C1*, and “C1 or C2 only” frequencies for individuals with resolved and persistent HCV infection. An odds ratio (OR) larger than one indicates a protective association. For genetic factors in *bold type*, the lack of the factors was considered for the analysis. Only genetic factors having frequencies in the range (~17–83 %), where significant difference was potentially detectable, were included in the analysis (one exception being *MHC-B C1/C1*, with a frequency of 12.5 %). Significance was assessed using a two-tailed Fisher Exact test. *NS* not significant, *NA* not available