Expression of iKIR-HLA-Cw in patients with inflammatory bowel disease

Huixia Zhang[1], Shuman Liu[2], Zhanju Liu[3], Jichang Li[1,*]

[1]*Department of Gastroenterology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan* 450052*, China;* [2]*Department of Histology and Embryology, Basic Medical College, Zhengzhou University, Zhengzhou, Henan* 450052*, China;* [3]*Department of Gastroenterology, The Second Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan* 450014*, China*

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Abstract

Objective. To investigate the distribution of inhibitory killer cell immunoglobulin-like receptor (iKIR) and its ligand human leukocyte antigen C locus (HLA-Cw) in patients with inflammatory bowel disease (IBD), and explore whether iKIR/HLA-Cw combinations are associated with IBD susceptibility. *Methods.* The iKIR in 100 patients with ulcerative colitis (UC), 52 patients with Crohn's disease (CD) and 106 randomly ethnically matched healthy controls was phenotyped by sequence-specific primer PCR (PCR-SSP). HLA-Cw was phenotyped by CLOCUS SSP UNITRAY®. The combination of HLA-Cw and its corresponding iKIR in individual was analyzed subsequently. *Results.* The KIR2DL1 and KIR2DL3 gene phenotype frequencies in UC patients were 0.710 and 0.620 respectively, both significantly lower than those in healthy controls, and the KIR2DL1 gene phenotype frequency in CD patients was 0.731, significantly lower than that in healthy controls. KIR2DL1-HLA-C2 combination in patients with UC and CD were 0.380 and 0.404 respectively, both significantly lower than that in healthy controls. *Conclusion.* The susceptibility to IBD is associated with decreased KIR2DL1-HLA-C2 combination. [Life Science Journal. 2008; 5(4): 17 – 22] (ISSN: 1097 – 8135).

Keywords: killer cell immunoglobulin-like receptor; human leukocyte antigen; inflammatory bowel disease; natural killer cell

1 Introduction

Crohn's disease (CD) and ulcerative colitis (UC), also known as inflammatory bowel disease (IBD), are characterized by chronic inflammation of the gastrointestinal tract. While the causes of IBD are unknown, it is thought that inflammation results from an inappropriate chronic activation of the innate and adaptive mucosal immune systems in a genetically susceptible host[1] and that enteric microflora plays a central role in the initiation and maintenance of disease. Epidemiological and genetic research has provided firm evidence for the existence of genetic determinants of susceptibility to IBD, and raised expectations that the identification of IBD susceptibility genes may lead to a clearer understanding of pathogenesis, and ultimately better treatment. Genome-wide screening in UC has demonstrated replicated linkage to several chromosomal regions, including regions on chromosomes 6, and 19 termed IBD3 and IBD6, respectively[2,3]. The killer cell immunoglobulin-like receptor (KIR) gene family is located within the IBD6 linkage region at chromosome 19q13.4. KIRs are expressed on natural killer cells and subsets of $\gamma\delta^+$ T cells and memory and effector $\alpha\beta^+$ T cells (usually $CD8^+$ T cells and some $CD4^+$ T cells), and can be divided on functional grounds into inhibitory and activating receptors. KIR possess a variety of inhibitory and activating receptors that upon recognizing and binding to their HLA class I ligands on target cells, regulate activation and inhibition of NK cell responses. Inhibitory receptors with specificity for human leukocyte antigen (HLA) class I appear to be important mediators of self-tolerance for NK cells. iKIR with the appropriate HLA ligand induces inhibitory signal depending on the presence of intracellular immunoregulatory tyrosine-based inhibitory motifs, pro-

*Corresponding author. Email: lijichang99@126.com

tecting self cell from the attack of natural killer (NK) cell.

HLA class I are encoded on chromosomes 6p21.3. HLA class I molecules are expressed on the surface of nearly all nucleated cells and mediate interactions with T lymphocytes, which recognize peptides bound to class I molecules, and NK cells, which recognize particular allelic forms of class I molecules. As with their MHC class I ligands, the population diversity and rapid evolution of the KIR genes strongly suggests that they are under pathogen-mediated selection[4]. It has been documented that alterations in the expression of KIR repertoire on NK cells as well as its corresponding ligand are associated with autoimmune diseases[5,6]. Combinations of KIR and HLA class I ligand variants that reduce NK cell inhibition have been shown to increase susceptibility to autoimmune diseases as well as resistance towards infections[6–10]. HLA C locus (HLA-Cw) molecules belong to classical HLA class I, and KIR-HLA-Cw molecule interactions are important for KIR recognizing target cells. Recent studies suggest that IBD may share an underlying pathogenesis with other autoimmune diseases. A much higher risk for rheumatoid arthritis (RA), ankylosing spondylitis (AS), psoriatic arthritis (PsA) and multiple sclerosis (MS) was observed in IBD patients compared with controls[11]. This promotes us to investigate whether KIR gene and HLA-Cw alleles play roles in the regulation of autoimmunity for IBD. In this study, we investigated the distribution of iKIR and its ligand HLA-Cw in patients with IBD, and explore whether iKIR-HLA-Cw combinations are associated with IBD susceptibility.

2 Materials and Methods

2.1 Subjects

The study included 100 patients with UC, 52 patients with CD and 106 randomly geographically and ethnically matched healthy controls. The patients were recruited from the Second Affiliated Hospital of Zhengzhou University in China. IBD was diagnosed and classified according tostandard clinical, endoscopic or radiological, and histological criteria criteria. Median age at diagnosis of IBD was 41.3 years old (range 16 – 81). Detailed demographic and clinical information were obtained and whole blood was taken for DNA extraction and subsequent genetic analysis. An ethnically and gender-matched group of healthy controls was randomly selected from blood donors and general practice registers, with ages ranging from 17 to 79 years, with an average of 41.5 years.

2.2 DNA preparation

Genomic DNA was extracted from whole blood using a Relax Gene Blood DNA system (TIANGEN, China). Yielding high molecular DNA validated for genotyping purposes.

2.3 KIR genotyping

The KIR genotyping was performed by means of sequence specific primer PCR (PCR-SSP) in all the recruited subjects for the following iKIRs: KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5, KIR3DL1, KIR3DL2 and KIR3DL3. The sequence specific polymorphism primers used for the detection of iKIR loci were based on primer sites that have been previously described[12–14]. The sequences and positions of the different primers were listed in Table 1. All reactions contained internal control primers specific for human growth hormone. All primers were synthesized and validated by Sangon Biological Engineering Technology and Service Co. Ltd (Shanghai, China). Each experiment included negative control reactions containing distilled water as a template. KIR specific primers were used at a final concentration of 0.4 μM. Amplifications of KIR genes were performed in 25 μl reactions, 0.75 U Taq Polymerase (Promega, USA), 0.2 mM dNTPs (Takara, Japan), 2 mM $MgCl_2$, 50 ng/μl of genomic DNA. All were under the following conditions except for KIR2DL2 and KIR3DL2: initial denaturation at 95 ºC for 2 minutes, then 30 seconds at 95 ºC, 30 seconds at 65 ºC, 1.5 minutes at 72 ºC for 30 cycles, and a final extension of 5 minutes at 72 ºC. KIR2DL2 and KIR3DL2 was carried out under the following conditions: at 95 ºC for 2 minutes, then 30 seconds at 95 ºC, 30 seconds at 63 ºC, 1.5 minutes at 72 ºC for 30 cycles, and a final extension of 5 minutes at 72 ºC. Amplification products were analyzed by the presence or absence of an electrophoretic specific band in 2% agarose gel stained with ethidium bromide in 0.5 × TBE buffer and visualized by ultraviolet light.

2.4 HLA genotyping

HLA-Cw genotyping of all subjects were performed using the HLA-C LOCUS SSP UNITRAY® typing kits (Invitrogen, USA). Low resolution PCR-SSP sets was used for typing according to the manufacturer's instructions. The kit was commercialized which used 23 pairs of locus-specific primers, and one negative control by the manufacturer. Alleles were assigned using the Pattern Matching Program provided by Dynal (Invitrogen, USA).

2.5 Statistical analysis

Gene frequencies were calculated with the formula of Hsu *et al*[13]. Briefly, observed frequencies of KIR genes were determined by the ratio of gene presence within the

Table 1. iKIR gene primer sequence

iKIR gene	Sense (5' – 3')	Antisense (5' – 3')	Amplication (bp)
KIR2DL1	CTGTTACTCACTCCCCCTATCAGG	AGGGCCCAGAGGAAAGTCA	1770
KIR2DL2	CATGATGGGGTCTCCAAA	CCCTGCAGAGAACCTACA	1808
KIR2DL3	CCTTCATCGCTGGTGCTG	CAGGAGACAACTTTGGATCA	812
KIR2DL4	CCCCTCAACAGATACCAGCGTGTG	GCAGGCAGTGGGGACCTTAGACA	271
KIR2DL5	GCTCTTCTTTCTCCTTCATTGCTGC	GCAGGCAGTGGGGACCTTAGACA	1025
KIR3DL1	AAGACACCCCCTACAGATACCATCT	GCAGGCAGTGGGGACCTTAGACA	277
KIR3DL2	TCCTGTCTGCCCGGCCCAGCA	ACCCAGTGAGGGAGTGTG	228
KIR3DL3	AACACGGAACTTCCAAATGCTGAGCG	GCAGGCAGTGGGGACCTTAGACA	243
HGH	GCCTTCCCAACCATTCCCTTA	TCACGGATTTCTGTTGTGTTT	429

population to the total population number. KIR gene frequencies were estimated by the formula: gene frequency $= 1 - (1 - f)^{1/2}$, where f stands for the observed KIR gene frequency in the population. Associations of presence or absence of single HLA-C and KIR alleles and receptor-ligand pairings were assessed using chi-square tests from two-by-two contingency tables. The statistical package SPSS, version 10.0 was used for analyses.

3 Results

3.1 KIR phenotypic frequency and genotypic frequencies in patients and control subjects

We genotyped both cohorts for the presence or absence of all 8 iKIR genes and their HLA-Cw ligand epitopes. The phenotype frequencies of iKIR loci and HLA-Cw epitopes were displayed in Table 2. The framework genes KIR2DL4, KIR3DL2 and KIR3DL3 were present in all individuals. The KIR2DL1 and KIR2DL3 gene phenotype frequencies in UC patients were 0.710 and 0.620 respectively, both significantly lower than those in healthy controls ($P = 0.001$). The KIR2DL1 gene phenotype frequency in CD patients was 73.1%, significantly lower than that in healthy controls ($P = 0.007$), and none of the other iKIR differed significantly. iKIR PCR-SSP genotyping were shown in Figure 1. An example of HLA-Cw typing was shown in Figure 2.

3.2 Decreased frequency of HLA-C group 2 alleles in individuals with UC

According to previous reports, HLA-C was divided into group 1 or group 2 depending on whether there was an asparagine or lysine present at position 80 of the 1 domain. In this study, HLA-C2 phenotype frequencies were significantly lower in UC group than in healthy controls ($P = 0.02$). The HLA-C1 phenotype frequencies in IBD patients did not significantly differ from those in healthy controls.

Table 2. Phenotype frequencies of iKIR and HLA-Cw in UC and CD

item	UC ($n = 100$)	CD ($n = 52$)	Controls ($n = 106$)
iKIR			
KIR2DL1	0.710*	0.731**	0.896
KIR2DL2	0.150	0.154	0.170
KIR2DL3	0.620*	0.769	0.821
KIR2DL4	1.000	1.000	1.000
KIR2DL5	0.230	0.192	0.198
KIR3DL1	1.000	0.962	0.972
KIR3DL2	1.000	1.000	1.000
KIR3DL3	1.000	1.000	1.000
HLA-Cw			
HLA-C1	0.830	0.808	0.811
HLA-C2	0.520#	0.519	0.641

vs. healthy controls: *$P = 0.001$,** $P = 0.007$, #$P = 0.02$.

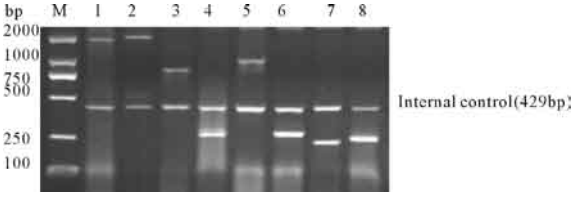

Figure 1. iKIR genotyping: Positive for the following iKIR from Lane 1 to Lane 8: KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5, KIR3DL1, KIR3DL2, KIR3DL3; M: molecular mass marker.

3.3 The combination of HLA-Cw and iKIR in IBD

Receptor effector function depend on the presence of corresponding ligand. Individuals who express simultaneously iKIR and its corresponding HLA-Cw ligand have

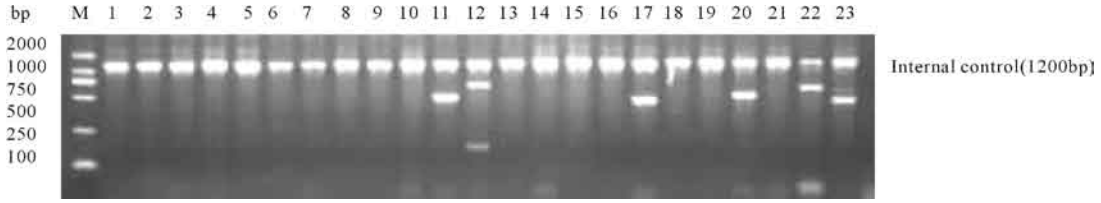

Figure 2. Gel electrophoresis of PCR-SSP products of HLA-Cw*08/HLA-Cw*15 heterozygotes. M: Molecular mass marker.

Table 3. The phenotype frequency of iKIR-HLA-Cw in patients with IBD and healthy controls

iKIR-HLA-Cw	UC (n = 100)	CD (n = 52)	Controls (n = 106)
2DL1$^+$/HLA-C2$^+$	0.380*	0.404**	0.575
2DL1$^+$/HLA-C2$^-$	0.330	0.327	0.321
2DL1$^-$/HLA-C2$^+$	0.1000	0.115	0.066
2DL1$^-$/HLA-C2$^-$	0.190$^{\#}$	0.154$^{\#\#}$	0.038
2DL2 and/or 2DL3$^+$/HLA-C1$^+$	0.630	0.654	0.698
2DL2 and/or 2DL3$^+$/HLA-C1$^-$	0.060	0.135	0.132
2DL2 and 2DL3$^-$/HLA-C1$^+$	0.200	0.154	0.113
2DL2 and 2DL3$^-$/HLA-C1$^-$	0.110	0.058	0.057

vs. healthy controls: $^*P = 0.05$,$^{**}P = 0.042$, $^{\#}P = 0.001$, $^{\#\#}P = 0.023$.

inhibitory function. From Table 3, we found that individual inhibitory KIR-HLA genotypes differ significantly between IBD patients and controls. KIR2DL3-HLA-C1 is dominant in IBD patients and controls. The phenotype frequency of KIR2DLl-HLA-C2 combination was 38% (UC) and 40.4% (CD), respectively. The inhibitory KIR2DL1-HLA-C2 genotypes were less frequently present in UC (P = 0.005) and CD (P = 0.042) patients than control subjects. 19.0% UC patients and 15.4% CD patients lack KIR-HLA-C2 (P = 0.001, P = 0.023, respectively), significantly different from the controls. The phenotype frequencies of KIR2DL2/KIR2DL3 and HLA-C1 combination in IBD patients did not significantly differ from those in healthy controls.

4 Discussion

NK cell effector function is perceived to be an important first line of innate immunity across viral, bacterial and parasitic infections, as well as forming an important bridge for activation of the adaptive immune response[15]. NK cell function is regulated by the balance between activating and inhibitory receptors in a majority of cases, interacting with HLA class I molecules. KIRs on NK cells and their ligands, HLA class I molecules, play an essential part in this tight regulation. Individual differences in NK cell interactions are dependent on combinations of variable KIR and HLA class I gene products[16]. Different receptor-ligand interactions may result in altered NK cell mediated immunity against pathogens[17]. An emerging body of evidence is accumulating to suggest that KIRs and HLA class I ligands contribute to the pathogenesis of diverse kinds of autoimmune diseases such as RA[18], AS[19], PsA[9], systemic lupus erythematosus (SLE) and Scleroderma[20].

The human KIR gene family contains 15 genes and 2 pseudogenes that are closely linked on chromosome 19q13.4. The number of KIR genes in the genome of any given individual varies within the population. KIRs contain either two or three immunoglobulin-like domains with either long (2DL, 3DL) or short (2DS, 3DS) cytoplasmic tails[21]. The presence of a long cytoplasmic tail (L) with immune tyrosine based motifs (ITIM) permits the transduction of inhibitory signals and characterizes the inhibitory KIR (2DL, 3DL), which inhibit NK and cytotoxic T lymphocyte (CTL) mediated lysis of target cells expressing appropriate HLA class I ligands. In contrast, the presence of a short cytoplasmic tail (S) corresponds to the activating or non inhibitory KIR (2DS, 3DS), which may enhance cytolysis of target cells. KIR binding motifs to HLA-C are defined by a dimorphism in the α1 domain of the HLA-C heavy chain. HLA-C molecules with Ser77 Asn80 are in Group C1 (-Cw1,-Cw3, -Cw7, -Cw8, -Cw12, -Cw14 and -Cw1601). HLA-C molecules with Asn77 Lys 80 are in Group C2 (-Cw2, -Cw4, -Cw5, -Cw6, -Cw15, -Cw1602, -Cw17 and -Cw18)[6]. HLA-C1 allotypes are ligands for KIR2DL2/3 (inhibitory) and possibly KIR2DS2 (activating), whereas HLA-C2 allotypes are bound by KIR2DL1 (inhibitory) and KIR2DS1 (activating).

KIR and HLA-C molecules play an essential role during the development of NK cells, because functional maturation of NK cells requires specific interaction with MHC class I molecules. For example, NK cells that express MHC class I-specific inhibitory receptors have been found to be functionally reactive, but NK cells that lack MHC class I-specific inhibitory receptors are hyporeactive to the same stimuli and do not exhibit cytotoxicity to

MHC class I-deficient target cells[22,23]. HLA class I and KIR genes are encoded on distinct chromosomes 6p21.3 and 19q13.4 and inherited independently[24]. The independent segregation of HLA and KIR genes raises the possibility that any given individual can express the receptor or the ligand only, or both receptor and ligand, so some individuals lack specific KIR-HLA receptor-ligand pairings. Both the KIR receptor and its specific HLA ligand must be present in order to regulate NK cell activity, such that one without the other is functionally inert. It would be interesting to investigate the corresponding HLA class I ligands to determine whether their KIR interaction is important in disease progression. In this regard, we lay particular emphasis on the key polymorphic ligand/receptor to determine the relative contribution of inhibitory KIR2D genes and HLA-C alleles to IBD susceptibility.

In the present study, the KIR2DL1 and KIR2DL3 gene phenotype frequencies were significantly lower in UC patients than in healthy controls, and the KIR2DL1 gene phenotype frequency was significantly decreased in CD patients as compared with healthy controls. KIR2DL1-HLA-C2 combination were significantly decreased in patients with UC and CD as compared with healthy controls. Regarding KIR2DL2/KIR2DL3-HLA-C1, we found no significant differences in gene frequencies in IBD patients as compared with healthy controls. Our results showed that IBD (UC and CD) susceptibility is mainly associated with a decrease in inhibitory KIR2DL1-HLA-C2 combinations.

The mechanisms by which KIR and HLA class I ligand genotypes influence susceptibility to autoimmune diseases are not clear[25]. However, as NK cells are constitutively inhibited by self-HLA class I molecules, most studies emphasize that an imbalance of KIR and/or HLA class I ligands resulting in reduced inhibition or increased activation is the critical determinant[6]. Mono-KIR NK cells without the corresponding ligands in the individuals and NK cells lacking all inhibitory receptors behave self-tolerant. Reduced combinations of KIR2DL1 and HLA-C2 in patients with UC and CD probably make NK cell and some killer T cell lack sufficently inhibitory signal, attack self cell, and finally result in autoimmune disease. Several of the genetic studies have suggested a model whereby inhibition of NK cells by some KIR-HLA combinations is stronger than others. In this model, the inhibition by KIR2DL1-HLA-C2 is strongest, followed by KIR2DL2-HLA-C1, and finally KIR2DL3-HLA-C1[6,7,26,27]. Accordingly, weaker inhibitory interactions result in greater NK cell activation and better protection from virus infection, or greater susceptibility to autoimmunity.

So KIR2DL2/L3-HLA-C1 should be more easily overridden by activating signals than a stronger inhibitory interaction such as KIR2DL1-HLA-C2[7]. Environmental insults affecting the HLA-C1 ligand, such as infection-mediated down regulation or masking of the iKIR-binding motif by a bound viral peptide, could promote the breakdown of self-tolerance and trigger autoimmunity in individuals carrying just one weakly iKIR + HLA combination. Interestingly, in our study, the majority of individuals in UC and CD possessed the weaker KIR2DL2/3-C1 as the only possible regulatory combination, lacking C2 ligand for KIR2DL1.

5 Conclusion

In this study, The susceptibility to IBD is mainly associated with a decrease in inhibitory KIR2DL1-HLA-C2 combinations. The decrease of combination KIR2DL1 and HLA-C2 may reduce the activating threshold of NK cells and CTL, enhance the cytolytic activity of lymphocyte and promote its multiplication, and finally lead to immune response to self-organizations in IBD. KIR and HLA are highly polymorphism, interacting with eath other, various KIR and HLA comprise various compound. It is important to explore various KIR-HLA compound in IBD.

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