The deleterious effect of the HLA-C1/KIR2DL3 receptor-ligand combination on Chinese HIV Patients

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Abstract

Background

Among Chinese HIV-infected patients we assessed the impact of different KIR-HLA combinations on the viral set point levels, CD4/CD8 ratios and plasma soluble CD14, all of which are risk factors for the prognosis of HIV patients.

Methods

Peripheral blood samples were collected from newly diagnosed HIV-infected patients who were treatment naive, and processed according to the study design. Participants were selected according to the inclusive criteria and their clinical data and other demographic information were recorded. The genomic DNA of the host was extracted from the whole blood samples, and the KIR genotyping was performed by sequence specific primer amplification (PCR-SSP); the HLA genotyping was performed by sequence analysis (PCR-SBT); and the HLA-KIR genotyping and combination information were obtained.

Results

In China, the distribution of KIR genotypes in HIV patients was similar to that of KIR genotypes in people without HIV infection; the baseline HIV level in patients with HLA-C1+/KIR2DL3+ background was significantly higher than that of HLA-C1+/KIR2DL3-(4.34 log10 copies/ml v.s 3.72 log10 copies/ml, P=0.02); the baseline CD4/CD8 ratio of HLA-C1+/KIR2DL3+ was significantly lower than that of HLA-C1+/KIR2DL3-(0.33 v.s 0.56, P=0.02). The plasma soluble CD14 level at baseline was significantly lower in HLA-C1+/KIR2DL3- infected persons than that of HLA-C1+/KIR2DL3+ (P=0.03).

Conclusions

NK cells may affect the viral set point and host immune status in HIV-infected patients through the combination of KIR molecules and their corresponding ligand HLA, thus affecting the prognosis of HIV infection.
Background

The effect of the innate immune system is directly related to the prognosis of HIV infection[1]. Natural killer (NK) cells are important cellular subsets of the innate immune system[2]. The interaction between the NK cell immunoglobulin like receptor (KIR) and its ligand type I human leukocyte antigen (HLA) is known to modulate the activity of NK cells. Previous studies[3, 4] have shown that KIR and its ligands, type I HLA genes, are extremely rich in genetic polymorphisms. A particular kind of HLA molecules is only identified by specific KIRs: the combination of HLA-C1 and KIR2DL2/2DL3[5, 6] and the match of HLA-Bw4 and KIR3DL1/S1[7, 8]. Clinical studies[9, 10] have found that HIV infected patients with different HLA/KIR combinations have different infection outcomes; for example, in patients with the combination of HLA-Bw4 and KIR3DS1, their progression to AIDS is slower than other patients. However, in the study of Carrington M et al[11], it suggested that the polymorphisms of KIR and its ligand HLA differed between regions and races. At present, there has been little research focused on the study of the combination of KIR-HLA affecting the prognosis of HIV infected individuals in China.

Previous studies[12, 13] have found that the level of viral set point directly affects the rate of progression of HIV. It should also be emphasized that the baseline CD4/CD8 ratio is an important indicator for the progression of HIV[14–16]. For patients with baseline CD4/CD8 >1, their rate of progression to AIDS is much slower[14]; otherwise after effective antiviral therapy patients’ CD4/CD8 ratio remains below 0.5, the occurrence of non AIDS related diseases, such as cardiovascular disease or cancer risk has been shown to significantly increase[15]. Furthermore, the expression level of certain inflammatory markers, such as elevated soluble CD14 levels, could also predict the morbidity and mortality of non-AIDS disease among HIV-infected patients[17, 18]. Because the previous studies[19–21] demonstrated that the combination of KIR-HLA may
affect the prognosis of HIV infection in Caucasians or Africans. We hypothesized that, specific KIR and HLA combinations among Chinese HIV infected patients may have an impact on the viral set point, CD4/CD8 ratio, and the soluble CD14 levels.

Methods

Study subjects

Inclusion criteria

1. From December 2015 to May 2016 in the clinic of Shanghai Public Health Clinical Centre and will be able to follow up on schedule
2. HIV infection has been confirmed by the test of Western Blot
3. No previous use of ART
4. Age >18 years
5. Ability to sign informed consent.

Exclusion criteria

1. Patients with opportunistic infections such as pulmonary tuberculosis, cryptococcal meningitis, severe pulmonary infection
2. Due to personal reasons reluctant to start antiviral treatment
3. Does not match the definition of HIV set point.

The definition of HIV set point

1. 5–12 months after the estimated infection date; or 2. with estimated infection date more than one year, CD4 cell counts must have been more than 350 cells/mm³ and no symptoms of AIDS.

We reviewed participants’ demographic characteristics (gender, age, infection routes, marital status, HIV confirmed time) and disease relevant information (CD4 counts, HIV-RNA load, CD4/CD8 ratio) from Hospital Information System (HIS). Blood samples for HIV-RNA virus load and CD4+ T-Cell count measurement were analyzed with COBAS TaqMan (Roche,
Switzerland) and CYTOMICS-FC500 respectively at the Shanghai Public Health Clinical Centre affiliated to Fudan University. HIV-1 genotypic and ART drug resistance analyses were performed according to the literature published elsewhere[22].

Healthy volunteers

In May 2016, 19 healthy volunteers (15 males and 4 females) were recruited in Shanghai Public Health Clinical Center. After informed consent, peripheral blood 20ml was collected. There was no significant difference in demographic data such as age and gender between healthy controls and those with HIV infection(P>0.05).

KIR and HLA genotyping and sCD14 determination

The determination of KIR genotype was based on the PCR and gel-electrophoresis which have been reported elsewhere[23].The determination of HLA genotype was based on the PCR and sequence based typing which have been reported elsewhere[24]. The plasma biomarker of gut bacterial translocation LPS/LBP and the marker of immune activation sCD14 were measured by ELISA. Their plasma concentrations were compared between HIV infected patients and healthy controls.

Statistical Analysis

Continuous variables were described using median and interquartile ranges (IQR) while categorical variables were described by numbers and percentages. The Fisher Exact test was used for categorical variables and Mann-Whitney test for continuous variables(chi square test and t test were not suitable for these data according to the principle of statistics). All hypothesis testing was 2-sided with a level of α = 0.05. Data analysis was conducted using IBM SPSS version 19.0 (IBM SPSS, Inc., Armonk, NY, USA), and the figures were constructed using GraphPad Prism version 5.0.

Results

Most of the patients were transmitted by sex contact and the median age was 32 years.
Among the distribution of HIV subtypes in all 116 cases, the largest number of subtypes was CRF-01AE (68 cases), followed by subtype B and C. A total of 10 HIV patients were tested drug-resistance positive. According to the results of sequence alignment, they were divided into three groups: low, moderate and high drug resistance. Among them, 2 were resistant to protease inhibitors, 7 were resistant to non-nucleoside reverse transcriptase inhibitors and 1 was resistant to nucleoside (see Table 1).

Among 116 subjects, the gene of DRB1 was made as internal controls, while all of the 116 patients were 2DL4, 3DL2 and 3DL3 positive, respectively. The number of 2DS3 positivity was 20. The percentages of 2DL4, 3DL2 and 3DL3 were 100%, 100% and 97% respectively. The rest of the KIR gene positive patients accounted for 17%—99% of the total subjects. See Figure S1 (Supplementary Figure S1). There were 83 alleles of HLA-B in the 116 HIV-infected individuals. Only allele frequencies above 1% are shown in Supplementary Figure S2. The HLA-B allele distribution was more dispersed among the infected individuals. The highest frequency HLA-B13:01 accounted for 6.9%, while HLA-B46:47 accounted for 1.29%. The distribution of HLA-C alleles was also relatively scattered. There were 76 HLA-C alleles in the 116 HIV-infected persons. In Supplementary Figure S3, only allele frequencies above 1% were typed. Among them, the highest frequency was HLA-C01:02 accounting for 12.07%, while HLA-C12:30 accounted for 1.29% (see Supplementary Figure S3).

Among all the subjects, HLA-Bw4 carriers were the most prevalent one (63.8%). HLA-C1 carriers accounted for 93.1% of all subjects, while homozygous HLA-C2/C2 accounted for only 7% of the total participants. Patients with the ligand-receptor combination of HLA-C1/KIR2DL3 have much higher HIV set point and lower ratio of baseline CD4/CD8. There was no significant differences between HLA-Bw4+/KIR3DS1+ and HLA-Bw4+/KIR3DS1- infected persons with respect to viral set point (P >0.05); patients with HLA-Bw6+/KIR3DS1+ or HLA-Bw6+/KIR3DS1- had no significant differences in viral set-point.
However, for patients with the HLA-C1+/KIR2DL3+ combination, viral set-point was significantly higher than that of HLA-C1+/KIR2DL3- (P < 0.05). (see Table 2). There was no significant difference in baseline CD4/CD8 ratios between patients with different HLA-Bw4/KIR3D combinations and HLA-Bw6/KIR3D pairing combinations. However, the baseline CD4/CD8 ratio of the HIV-infected persons with HLA-C1+/KIR2DL3- was significantly higher than those with HLA-C1+/KIR2DL3+ (P = 0.02)(see Table 3). The baseline viral load of the HLA-C1+/KIR2DL3+ group was significantly higher than that of the HLA-C1+/KIR2DL3- group (P = 0.02). However, with the initiation and continuation of anti-retroviral therapy, in both groups, the viral load rapidly decreased, as expected, and the difference in reduction gradually narrowed. Indeed, by the end of the 6 month treatment period, there was no difference of viral load between the two groups(see Figure 1). From the baseline, there was a significant difference in the CD4/CD8 ratio between the two groups (P = 0.02). With the antiretroviral treatment, the CD4/CD8 ratio increased in both groups, while the HLA-C1+/KIR2DL3+ group had a higher CD4/CD8 ratio at 6-months treatment, but there was no significant difference between the two groups (P = 0.379). The plasma LPS concentration of HIV-infected patients at baseline was significantly higher than that of healthy controls (P < 0.001), and the plasma sCD14 level was also significantly higher than that of healthy people, but there was no significant difference in plasma LBP level between the two groups(see Figure 2). The plasma soluble CD14 level at baseline was significantly lower in patients with HLA-C1+/KIR2DL3- than in those with HLA-C1+/KIR2DL3+ (P = 0.03). There was no significant difference in soluble CD14 levels based on either age or sex in the 2 groups. In addition, the baseline plasma LPS of HLA-C1+/KIR2DL3- HIV infected patients was lower than that of HLA-C1+/KIR2DL3- HIV infected patients, and the plasma LBP level of the former was higher than that of the latter, but the difference was not statistically significant(see table
Discussion

KIR and HLA molecules are known to effect the pathogenesis and prognosis of many diseases because of their regulatory roles on NK cell immunity[25–28]. According to the results of HLA genotyping, the frequencies of HLA-Bw4 and HLA-C1/C2 genotyping are basically the same as those of previous HLA-B/C genotyping researches based on Eastern Chinese population[29]. Furthermore, the frequency of KIR is also very close to the results of non-HIV patients in Eastern China[29, 30].

The HIV set point level of the infected patients directly affects the rate of disease progression. According to the previous studies[31–34], the level of HIV set point in this study was defined as 5–12 months after the anticipated infection or the anticipated infection more than 1 year and CD4 >350/UL without initiating anti-HIV treatment. As for the effector of inhibitory KIR on NK cell, the role of HLA-A and HLA-B is weaker than HLA-C[35]. In this study, it was found that the HIV infected people with KIR2DL and its ligand HLA-C1 gene, their set points were significantly different from others without the combination of KIR2DL/HLA-C1. Patients with the combination of HLA-C1+/KIR2DL3+ had significantly higher levels than those with HLA-C1+/KIR2DL3-. These results are consistent with the previous clinical cohort study in Thailand[19]. In our study, there was no significant difference between the KIR3DS1+/HLA-Bw4+ group and the KIR3DS1-/HLA-Bw4+ group with HIV infection. This results contradicted the previous studies[36, 37] which reported that patients with the combination of KIR3DS1+/ HLA-Bw4+ showed slower progression of HIV-infection. The reasons may be as follows: previous studies focus on infection and control of the progress of the elite in the typical HIV (Elite controller) comparison between the KIR-HLA distributions, less involved in common HIV infection group. The current study presented here, through strict inclusion criteria and screening
process, we focused on the combination of HLA-KIR that affects the prognosis of HIV infection. Furthermore, the Nef protein of HIV will reduced the expression level of HLA-A and HLA-B while the HLA-C would maintain the original level[38-40]. As a result, the level of HLA-Bw4, as the ligand for NK cell activation receptor KIR3SD1 down regulation, which weaken the ability to activate NK immune effect and then decreased the function. Consequently, HLA-Bw4/KIR3DS1 protected effect reduced, while the expression of HLA-C1 and its ligand KIR2DL3 expression maintained after HIV infection. So compared with KIR2DL3+, the combination of HLA-C1/KIR2DL3- down regulate the inhibition of NK cell immune effector, and NK cells play the immune protected effects or delay the progression of HIV infection[41].

The clinical cohort study[19] in Thailand stated above found that the combination of HLA-C1/KIR2DL3- was one of the protective factors for delaying the progression of HIV infection. Our study found that both the viral set point and the baseline CD4/CD8 ratio support this conclusion. Since the level of immune activation is also an important factor affecting the progression of HIV infection, we further analyzed the effect of the HLA-C1/KIR2DL3 combination on the immune activation of HIV infection. We found that the plasma sCD14 level of infected persons with HLA-C1+/KIR2DL3+ was significantly higher than that of HLA-C1+/KIR2DL3- infected persons, and correspondingly, the HIV set point of the former was significantly higher than that of the latter. As suggested by Anas A et al[42], the level of sCD14 in plasma may be related to not only the increase of LPS and LBP caused by intestinal microflora translocation, but also the level of the plasma HIV load. Hence, the ability of HLA-C1+/KIR2DL3-to inhibit HIV replication is stronger than that of HLA-C1+/KIR2DL3+, thus weakening the immune activation induced by HIV infection. Consequently, this would result in making the plasma level of sCD14 becoming significantly lower in the former than in the latter. However, it should be noted that after
6 months of effective ART, although the viral load of HIV-infected patients decreased significantly, the plasma sCD14 level did not decrease accordingly, in fact, even increased significantly compared with the level at baseline. Maybe the plasma sCD14 is affected by many reasons while the HIV replication is just one of them.

There are some limitations in this study. Firstly, only 6 patients with KIR2DL3-, even though the differences in the study are statistically significant, but need to be cautious in the interpretation in reality. It is also needed to further expand the sample size. In addition, because of the small proportion of KIR molecules on the surface of CD8+ T lymphocytes, in our study we only discuss about the NK cell and the potential effects of CD8+ T cell can not be excluded from this study. This is also the limitation of this study.

Conclusions

Among HIV infected persons, the ones with the combination of HLA-C1+/KIR2DL3+ have much higher HIV set point and lower baseline CD4/CD8 than patients with the combination of HLA-C1+/KIR2DL3-. The baseline sCD14 levels in HIV-infected people with HLA-C1+/KIR2DL3+ receptor ligand combination is significantly higher than those with HLA-C1+/KIR2DL3-. This combination of HLA-C1+/KIR2DL3- may affect the immune activation of HIV infection by affecting the plasma level of sCD14.

List Of Abbreviations

HIV: human immunodeficiency virus; ART: anti-retroviral therapy; AIDS: acquired immunodeficiency syndrome; NK: Natural Killer; CDC: centers for disease control; IQR: interquartile range; IDU: injection drug use; HLA: Human Leukocyte Antigen; KIR: Killer Cell Immunoglobulin-Like Receptor; PCR: Polymerase Chain Reaction; SBT: Sequence Based Typing; LBP: Lipopolysaccharide Binding protein; LPS: Lipopolysaccharides; sCD14: Soluable CD14.
Declarations

Ethics approval and consent to participate
The study protocol was submitted and approved by the Shanghai Public Health Clinical Center Ethics Committee (Ethical number 2015-Y008-02). The written informed consent was obtained from all the study participants.

Consent for publication Not applicable.

Availability of data and materials All data generated or analysed during this study are included in this published article and its supplementary information files (S4 File).

Competing interests The authors declare that they have no competing interests.

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Authors’ contributors HZL, SYZ, JJS and JYS conceived and designed the study; JJS and LL, RFZ, JRW, YZS, JC, ZYW, YT, WS, TKQ, MYS, LQG, JC collected the data. JJS and JYS analyzed the data; JJS, JYS and SYZ, HZL interpreted the results; JJS and JYS wrote the first draft; JJS, JYS, HZL and SYZ contributed to the final version. All authors have read and approved the manuscript.

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Tables

| Demographics and clinical data          | All Subjects (n=116) |
|----------------------------------------|----------------------|
| Age                                    | 31.50 (27.25—39.75) |
| Gender                                 |                     |
| Male                                   | 113                  |
| Female                                 | 3                    |
| Infection routes                       |                     |
| Homosexual                             | 90                   |
| Heterosexual                           | 20                   |
| blood ✯                                | 1                    |
| undetermined                           | 5                    |
| HIV set point ✯                        | (Median, IQR)        |
| (Median, IQR)                          | 4.25 (3.72—4.70)    |
| Baseline CD4 ✯                         | (Median, IQR)        |
| (Median, IQR)                          | 327 (254—436)       |
| Baseline Ratio of CD4/CD8 ✯            | (Median, IQR)        |
| (Median, IQR)                          | 0.34 (0.25—0.51)    |
| HIV subtypes                           |                     |
| CRF-01AE                               | 68                   |
| B                                      | 41                   |
| C                                      | 7                    |
| Drug-resistance                        |                     |
| Yes                                    | 10                   |
| No                                     | 106                  |
including blood transfusion or intravenous drug user*: log_{10} copies/ml

IQR: interquartile range.

Table 2. Comparison of HIV set point between different ligand-receptor combinations of HLA/KIR

| HLA genotypes | KIR genotypes | HIV set point (log_{10} copies/ml) (Median, IQR ) | P value |
|---------------|---------------|---------------------------------------------------|---------|
|               | KIR(+)        | KIR(-)                                            |         |
| HLA-Bw4+  n=74| KIR3DL1 n=73  | 4.24(3.62-4.68) n=1 NA                            | --      |
|               | KIR3DS1 n=21  | 4.43(3.64-5.02) n=53 4.15(3.61-4.63)               | 0.22    |
| HLA-Bw4-80I+ n=35| KIR3DL1 n=35 | 4.33 (3.62-4.66) n=0 NA                            | --      |
|               | KIR3DS1 n=9   | 4.43(3.58-5.29) n=26 4.28 (3.61-4.66)               | 0.72    |
| HLA-Bw6+ n=97 | KIR3DL1 n=95  | 4.33(3.81-4.74) n=2 4.23 (3.85-4.60)                | --      |
|               | KIR3DS1 n=27  | 4.43(3.72-4.74) n=70 4.28 (3.83-4.75)               | 0.91    |
| HLA-C1+ n=108 | KIR2DL2 n=41  | 4.43(3.71-4.99) n=67 4.25 (3.78-4.66)               | 0.86    |
|               | KIR2DL3 n=102 | 4.34(3.77-4.74) n=6 3.72 (3.50-3.85)               | 0.02    |
|               | KIR2DS2 n=29  | 4.24(3.56-4.86) n=79 4.33(3.82-4.69)                | 0.28    |
| HLA-C2+ n=29  | KIR2DL1 n=28  | 4.06(3.49-4.33) n=1 NA                             | --      |

Not applicable IQR: interquartile range.

Table 3. Comparison of the ratio of baseline CD4/CD8 between different ligand-receptor combinations of HLA/KIR

| HLA genotypes | KIR genotypes | The ratio of baseline CD4/CD8 (Median, IQR ) | P value |
|---------------|---------------|---------------------------------------------|---------|
|               | KIR(+)        | KIR(-)                                      |         |
| HLA-Bw4+ n=74 | KIR3DL1 n=73  | 0.33(0.23-0.51) n=1 NA                      | --      |
|               | KIR3DS1 n=21  | 0.35(0.26-0.58) n=53 0.31(0.23-0.46)         | 0.31    |
| HLA-Bw4-80I+ n=35| KIR3DL1 n=35 | 0.31(0.23-0.51) n=0 NA                      | --      |
|               | KIR3DS1 n=9   | 0.29(0.21-0.46) n=26 0.32(0.23-0.52)         | 0.72    |
| HLA-Bw6+ n=97 | KIR3DL1 n=95  | 0.33 (0.25-0.49) n=2 0.52 (0.47-0.56)        | --      |
|               | KIR3DS1 n=27  | 0.34 (0.28-0.56) n=70 0.33 (0.25-0.48)       | 0.45    |
| HLA-C1+ n=108 | KIR2DL2 n=41  | 0.33(0.20-0.44) n=67 0.33 (0.25-0.51)        | 0.31    |
|               | KIR2DL3 n=102 | 0.33(0.25-0.49) n=6 0.56(0.39-0.84)          |        |
|               | KIR2DS2 n=29  | 0.35(0.19-0.53) n=79 0.33(0.25-0.49)         |        |
| HLA-C2+ n=29  | KIR2DL1 n=28  | 0.36(0.25-0.50) n=1 NA                      | --      |
Table 4. The comparison of baseline plasma LPS, LBP and sCD14 between HIV patients with HLA-C1+/KIR2DL3+ and HLA-C1+/KIR2DL3-

| Demographics and clinical data | All HIV patients (n=82) | HLA-C1+/KIR2DL3- (n=4) (Median, IQR) | HLA-C1+/KIR2DL3+ (n=78) (Median, IQR) | P value * |
|--------------------------------|-------------------------|--------------------------------------|--------------------------------------|-----------|
| Age (years median, IQR)       | 31.0 (27.8—39.0)        | 25.5 (21.3—32.8)                     | 31.5 (28.0—39.5)                     | 0.12      |
| Gender (Male/Female)          | 80/2                    | 4/0                                  | 76/2                                 | 1.0       |
| Baseline Viral load (log_{10} copies/ml) | 4.23 (3.71—4.66) | 3.72 (3.53—4.06)                     | 4.33 (3.71—4.69)                     | 0.10      |
| Baseline CD4 (cells/ul)       | 322 (251.8—426.5)       | 417.5 (262.5—462.3)                  | 317.5 (251.8—421.5)                  | 0.43      |
| Ratio of CD4/CD8 (median, IQR)| 0.32 (0.23—0.50)        | 0.56 (0.34—0.85)                     | 0.32 (0.23—0.49)                     | 0.07      |
| Baseline plasma LPS (ng/ml)   | 376 (218-539)           | 261 (141-700)                        | 378 (222-539)                        | 0.43      |
| Baseline plasma LBP (ug/ml)   | 10.94 (8.41-17.27)      | 14.68 (8.23-28.54)                   | 10.49 (8.32-17.15)                   | 0.51      |
| Baseline plasma sCD14 (ng/ml) | 2677 (2159-3514)        | 1711 (1353-2553)                     | 2723 (2174-3548)                     | **0.03**  |

*P value were calculated by Fisher exact test or Mann-whitney test

IQR: interquartile range.

Figures
Figure 1. Trends of HIV-RNA load from ART initiation to 6 months after.

| GROUP A | 0M  | 1M  | 2M  | 6M  | GROUP B | 0M  | 1M  | 2M  | 6M  |
|---------|-----|-----|-----|-----|---------|-----|-----|-----|-----|
| Number  | 102 | 101 | 101 | 82  | Number  | 6   | 6   | 6   | 5   |
| 1/4 IQR | 3.8 | 2.0 | 1.7 | 1.3 | 1/4 IQR | 3.5 | 1.6 | 1.3 | 1.3 |
| Median  | 4.3 | 2.3 | 2.0 | 1.7 | Median  | 3.7 | 2.0 | 1.7 | 1.7 |
| 3/4 IQR | 4.7 | 2.7 | 2.4 | 1.7 | 3/4 IQR | 3.8 | 2.1 | 2.1 | 1.7 |

Trends of HIV-RNA load from ART initiation to 6 months after.
Figure 2. The comparison of baseline plasma LPS, LBP and sCD14 between HIV patients and healthy volunteers.

Supplementary Files

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