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HIV-1 Infected Germans Have More Variations on Neck Region of DC-Specific Intercellular Adhesion Molecule-3-grabbing Nonintegrin than HIV-1 Infected Chinese

X. Li-Jun[1,2], N. H. Brockmeyer[3], A. Bader[3], Y. Hang-Ping[1], W. Zhi-Gang[4], W. Nan-Ping[1]

[1]Institute of Infectious Diseases, the first affiliated hospital of Zhejiang University, Hangzhou, P.R.China,
[2]Fuzhou Infectious diseases hospital, Fuzhou, P.R.China,
[3]Department of Dermatology, Ruhr-University, Bochum, Germany,
[4]CDC of Zhejiang Province, Hangzhou, P.R.China

Abstract
The C-type lectins DC-SIGN and DC-SIGNR bind and transmit HIV-1 depending on the tetramer formed by "neck" region and carbohydrate recognition domain (CRD). Recent studies indicated polymorphisms in neck region of DC-SIGNR were more frequent and associated with susceptibility to HIV-1 infection, whereas no similar trends were observed on DC-SIGN for rare variations of DC-SIGN among the researched subjects in those studies. In our present study, we found 9 genotype variations of DC-SIGN neck region among HIV infected Germans and 3 variations among HIV infected Chinese, respectively. Comparison of those variations between Chinese and Germans reveals strong ethnic discrepancies, and no significant evidence indicates those variations have resistance to susceptibility to HIV-1 infection.

Introduction

Dendritic cells (DCs) play a critical role in the initiation of immune response by virtue of their ability to capture and present antigens to T cells [1]. Although the precise mechanism by which DCs acquire Human Immunodeficiency Virus-1 (HIV-1) is not fully understood, migration of DCs from the periphery to the draining lymph nodes may enable CD4+ T cells to become infected [2]. DC-**S**pecific **I**ntercellular Adhesion Molecule 3 **G**rabbing **N**onintegrin (DC-SIGN), a mannose specific C-type lectin receptor on DCs, plays a vital role in this process by binding HIV-1-gp120 and facilitating DCs transport HIV-1 from the infection site to the secondary lymph nodes [3]. Another important molecule with similar implication on HIV-1 infection is DC-SIGNR (DC-SIGN related lectin, or L-SIGN), which shares 77% amino acid identity with DC-SIGN, and is expressed on endothelial cells in the liver, lymph nodes and placental capillaries [4]. DC-SIGN and DC-SIGNR are receptors for many pathogens, including HIV-1, Mycobacterium tuberculosis and Dengue viruses [5, 6].

Two structures on DC-SIGN/R, the carbohydrate recognition domain (CRD) and the neck region, play an important role in pathogen capture. The neck region in most cases is composed of seven tandem same repeat alleles, and every allele consists of 23 amino acids encoded by 69 nucleotides. The neck region forms a tetramer that determines whether the CRD can recognize and bind different microbes [7, 8] (Fig. 1). So variations of neck region may impact the stability of the tetramer and the ability of DC-SIGN/R to recognize pathogens.

Polymorphisms of DC-DIGN/R are associated with HIV-1 and other microorganism infections [9]. Certain variations of the DC-SIGN promoter greatly influence the efficiency of HIV-1, *M. tuberculosis*, and Dengue virus infection. Indeed, the -871G and -336A variants are shown to confer protection against tuberculosis [5]. The -336G variant is associated with strong protection against Dengue virus infection [6]. Individuals with the -336C variant are more susceptible to HIV-1 infection than individuals with the -336T variant [10]. The relationships between neck region of DC-SIGN/R and HIV-1 infection were discussed in recent researches. In vitro study[11]didn't support that DC-SIGNR homozygous repeats which were less than seven repeats displayed resistance to HIV-1 infection, yet, a cohort study[12] indicated that DC-SIGNR with

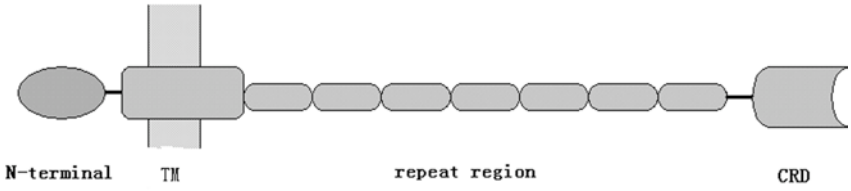

Fig. 1. Schematic Structure of DC-SIGN. DC-SIGN is composed of four parts: the C-terminal extracellular domain (CRD), the neck region, the transmembrane domain, and the N-terminal endocellular domain.

homozygous 7/7 repeat was found to be associated with an increased risk of HIV-1 infection, whereas the DC-SIGNR heterozygous 7/5 repeat was correlated with resistance to HIV-1 infection. However, HIV-1 infection was not only via DC-SIGNR, but also via DC-SIGN. Co-operations of DC-SIGN and DC-SIGNR may play an important role in HIV-1 infection, since polymorphisms of DC-SIGNR were possibly linkage disequilibrium with DC-SIGN.

Contrary to DC-SIGNR, DC-SIGN polymorphisms were rarely observed in HIV infected individuals up to date. Whether variations of DC-SIGN neck region had impact on HIV infection were still unknown. It was reasonable to investigate the potential correlation between discrepancies in the DC-SIGN neck region and susceptibility to HIV-1. The considerations for investigation of polymorphisms of DC-SIGN in HIV infected individuals are based on two reasons: i) whether the polymorphisms exist in HIV infected population or not, ii) if so, what roles they play in HIV/AIDS progress.

In our present study, we investigated the variations of DC-SIGN in HIV-1 infected Chinese and Germans and found more variations of DC-SIGN both in HIV infected German and Chinese.

Material and Methods

Samples: We collected 132 HIV-1 infected patients from HIV/AIDS research center, Ruhr University, Germany, and 119 HIV-1 infected individuals from China between 2004 and 2006. All the subjects were HIV primary infected individuals, and didn't receive any anti-virus therapy. With their authorization, five ml of blood was drained and preserved in EDTA-anticoagulant tubes from vein of subjects.

Reagents: DNA extract kit QIAamp DNA blood mini Kit and DNA polymerase Hot-Start Taq Master Mix Kit was purchased from Qiagen (Germany); Agarose was from Bio-West (Spain); Purelink PCR purification kit was from Invitrogen (USA), PMD-18T vector for T-A link reaction was from Takara (Japan).

DNA Extraction and PCR assay: 200 µl total blood were used for DNA extraction. Procedures were performed according to the handbook. The obtained DNA was suspended in 200 µl of distilled water. The DC-SIGN repeat region was amplified with sense primer 5'- AACAATCCAGGCAAGACG -3 and antisense primer 5'- TGCTCAGGCAGGGTCAGT-3' (designed from GenBank sequence AF209479). PCR was run for 30 cycles at 95 °C for 15 min, 94 °C for 30 sec, 60 °C for 30 sec, and 72 °C for 45 sec. PCR was completed at 72 °C for 7 min, and the samples were stored at 4 °C.

Analyzesing of DC-SIGN repeat region: PCR products were analyzed by 2% agarose gel electrophoresis. The products were then purified with a PCR purification kit, cloned into PMD-18T vector by T-A reaction, followed with a two-way sequencing (Applied Biosystems Prism sequencer 3730). Based on the results, nucleotide sequences of the DC-SIGN neck repeat region were genotyped.

Results

After amplifying the repeat region of DC-SIGN, 132 of HIV-1(+) samples of Germans, and 119 of Chinese samples were positive by PCR. Agarose gel electrophoresis indicated that variations of DC-SIGN neck region were observed in Chinese group and German. The results revealed there more frequency of variations in German group than in Chinese group (Fig. 2). For further analyzing, PCR products were purified and cloned into PMD-18T vector for sequence. There were 9 genotypes in German group and 3 genotypes in Chinese group. In German group, there were 8 with homozygous 8/8 repeats (8R), 90 with homozygous 7/7 repeats (7R), 6 heterozygous 8/7 repeats (8/7R), 2 heterozygous 8/6 repeats (8/6R), 11 heterozygous 7/6 repeats (7/6R), 1 heterozygous 7/5 repeats (7/5R), 10 homozygous 6/6 repeats (6R), 2 heterozygous 6/5 repeats (6/5R), 2 homozygous 6/6 repeats (6R), Whereas, there were 115 with homozygous 7/7 repeats (7R), 1 with homozygous 8/8 re-

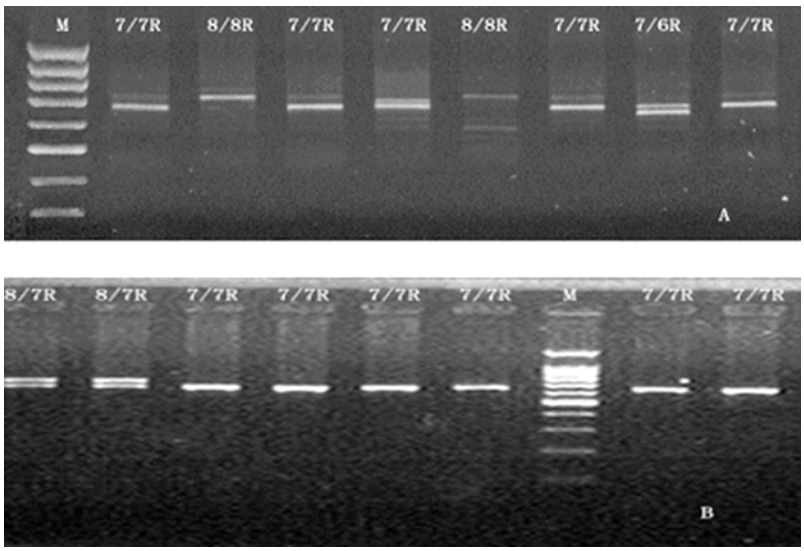

Fig. 2. Variations of DC-SIGN neck region in HIV infected individuals. A) Variations in HIV infected Germans; B) Variations in HIV infected Chinese. M is 100bp DNA marker.

Table 1. Distribution of genotypes of 8/8R, 8/7R and 7/7R between HIV infected Chinese and Germans.

			Group		Total
			Chinese	German	
Genotype	8/8R	Count	1	8	9
		Percent	0.8%	7.7%	4.0%
	8/7R	Count	3	6	9
		Percent	2.5%	5.8	4.0%
	7/7R	Count	115	90	205
		Percent	96.6%	86.5%	91.9%
Total		Count	119	104	223
		Percent	100%	100%	100%

Between Chinese and German group, the discrepancies were significant, P = 0.014

peats (8R),and 2 heterozygous 8/7 repeats (8/7R) in Chinese group.

Among both groups, homozygous 7/7 repeats were the most genotype(90 cases in German and 115 cases in Chinese),and were regarded as the wild type. Genotypes 8/6R(1.51%), 7/5R(0.76%), 6/5R(1.51%) and 5/5R(1.51%) were found, though rare, in German group, so we considered all those genotypes as one whole. The variations rate of these genotype were obviously higher in German (5.29%) than in Chinese(0%). Homozygous 6/6 repeats (6R) and heterozygous 7/6 repeats (7/6R) were 7.58% and 8.33% in German, respectively; nevertheless, these genotypes were not detected in Chinese. Pearson Chi-Square analyzing operated on SPSS11.0 was used to determine whether the distributions of genotypes 8/8R, 8/7R and 7/7R appeared both in German and Chinese were dependent on ethnical discrepancies. The statistic results revealed although the 8/8R and 8/7R had more frequencies in German than in Chinese (6.06% vs 0.84%, 4.54%vs2.52%, respectively), frequency of 7/7R in German was significant low than in China, indicating a strong ethnical discrepancies between German and Chinese (p = 0.014) (Table 1).

Discussion

The C-type lectin, DC-SIGN, is expressed on dendritic cells and on certain types of macrophages. DC-SIGN has a dual action on DCs. As an adhesion receptor, DC-SIGN supports initial DC–T cell interactions by binding to ICAM-3, and mediates the tethering of DCs to the endothelium via interaction with ICAM-2. As a pathogen-recognition receptor, DC-SIGN binds HIV-1 gp120 and facilitates the transport of HIV-1 from mucosal sites to the draining lymph nodes, where infection of T lymphocytes can occur [3, 4]. Peptide sequence and structural analyses indicate that DC-SIGN consists of four regions: the **carbohydrate recognition domain (CRD)**, consisting of 110 to 140 amino acid residues, the **neck region**, comprising seven 23-amino acid residue tandem repeat alleles, a **transmembrane region**, and an **endocellular domain** [7, 8]. The CRD and neck regions have the greatest influence on pathogen infection. Binding and transmission of HIV-1 and other pathogens by DC-SIGN is dependent on the presence of the CRD. The seven repeat alleles of the neck region form relatively stable tetramers, and variations in these alleles can affect tetramers formation.

A study of 835 Caucasian participants [13] indicated DC-SIGNR was highly polymorphic in the repeat region based on the number of repeats ranging from three to nine (alleles 3 to 9), whereas no similar polymorphisms in DC-SIGN were found. The polymorphisms of DC-SIGN were rare; alleles 7 was about 99.52% among population, though alleles 6 to 8 were occasionally observed. The association of D-SIGN/R and HIV-1 infection was studied in recent years [12, 14]. Those researches indicated that DC-SIGNR with heterozygous 7/5 repeat was likely to be correlated with resistance to HIV-1 infection, suggesting polymorphisms of DC-SIGNR may influence susceptibility to HIV-1.

For the reason of rare variations, DC-SIGN variations in HIV infected individuals were infrequent in previous reports [13, 14]. Here, in our present study, we first observed the variations in HIV infected people. The variant frequency of HIV infected German was higher than Chinese, suggesting strong ethnic origin differences. Huanliang Liu' works [14] revealed that individuals with heterozygous DC-SIGN repeat alleles were associated with reduced susceptibility to HIV-1 infection. Different from their results, we found many different allele combinations within the DC-SIGN neck region of HIV-1 infected individuals. The heterozygous repeat alleles could be observed both in HIV infected Germans and Chinese, and especially, more heterozygous repeat alleles appeared in Germans. At least our results do not support their conclusions.

Correlation between DC-SIGN structure and its function should be investigated. Luis M's research [15] concluded that polymorphisms of DC-SIGN repeat region were not relevant risk factors for developing tuberculosis in Northwestern Colombian individuals,

while, more works are needed to examine whether variation of allele combinations in vivo and in vitro is linked to the ability to recognize, bind and carry pathogens, and whether those different alleles play different roles on HIV/AIDS progresses.

In summary, the variation of DC-SIGN is correlated with ethnic discrepancies in HIV infected Chinese and German, and these variations of DC-SIGN are more frequent in German than in Chinese, suggesting heterozygous DC-SIGN repeat alleles may be irrelevant to HIV infection.

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Address for correspondence:
Wu Nanping
Institute of Infectious Diseases,
the first affiliated hospital of Zhejiang University,
No. 79, Qingchun Road,
Hangzhou,
Zhejiang Province,
310003, China
Tel.: ++86-571/87236579
Fax: ++86-571)87236582
E-mail: flwnp@yahoo.com.cn
flwnp@zju.edu.cn