The Gag Specific T lymphocyte Response of Chinese HIV-1 B/C Infectors at different stages

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Abstract: *Background* Gag is very important structural protein of human immunodeficiency virus type 1 (HIV-1) and could be detected in early infection. Specific T lymphocyte immune response was regarded as essential in controlling the production and infection. HIV-1 subtype B/C epidemic is speeding in China but limited data is available on the T cell responses covering Gag in the HIV-1 subtype B/C infectors at different stages. *Materials and Methods.*10 antiretroviral treatment（ART） naïve HIV-1 recombinant subtype B/C infectors with infected time in 1 year, 25 ART-naive infectors with infected time more than 3 years and 10 HIV-1-seronegative healthy individuals were enrolled. HIV-1-specific T lymphocyte responses were analyzed by an IFN-γ Elispot assay against 123 overlapping peptides spanning HIV-1 Gag protein in the present study. *Results* Gag-specific T lymphocyte responses of interferon-gamma secretion were identified in 8(80％) Chinese HIV-1 recombinant subtype B/C infectors with infected time in 1 year, the specific T lymphocytes are mainly targeted at five peptides: GAG7895 in Gag p17, GAG7912, GAG7951 in p24, GAG7979 in p7and GAG7992 in p6. Responses were identified in 17(68％) infectors with infected time more than 3 years, the specific T lymphocytes are mainly targeted at seven peptides: GAG7896 in Gag p17and GAG7911, GAG7912, GAG7917, GAG7923, GAG7924 and GAG7945 in p24. There was obviously positive correlation (P=0.0318，r^2 =0.269) between the magnitude of IFN-γ secretion T lymphocyte responses and plasma viremia in infectors infected time more than 3 years. The magnitude of response in infectors infected in 1 year was significantly higher than that in infectors with infected time more than 3 years (P=0.021). None of the seronegative healthy individuals gave the positive responses. *Conclusions* HIV-1 recombinant subtype B/C Infectors at different stages of diseases recognize different region of Gag. [Life Science Journal 2010;7(2):75-79]. (ISSN: 1097-8135).

Keywords: Human Immunodeficiency Virus (HIV)；Gag; Specific T lymphocyte Response；Elispot；IFN-γ

1 Introduction

Gag is very important structural protein of human immunodeficiency virus type 1 (HIV-1).The Gag specific T lymphocyte against HIV-1 could be detected in early infection[1]. Thus, the characterization of Gag specific T lymphocyte immune responses was regarded as essential in controlling the production and infection of HIV-1[2]. Molecular epidemiological studies in China have shown that the HIV-1 subtype B/C epidemic is speeding, but limited data is available on the T cell responses covering Gag at the single peptide level in the HIV-1 subtype B/C infection at different stages. The aim of this study was to use the overlapping peptides spinning the Gag to determine the scope and specificity of specific T lymphocyte immunity in Chinese HIV-1 subtype B/C infectors at different stages.

2 Materials and Methods

2.1 Materials

FITC-CD3 Ab,PE-CD4 Ab,APC-CD8 Ab and PerCP-CD45 Ab(Immunotech,USA),viral load reagents(Shenzhen, China), Peptides were obtained from the National Institute of Health AIDS Research and Reference Reagent Program (Cat # 8117 ， 7872-7994, USA). Ficoll(Sigma,USA),RPMI 1640, fetal calf serum,HEPES buffer(GIBCO,USA), Human IFN-γ ELISPOT kit (U-CyTech, Netherlands),flow cytometry (Beckman-Coulter,USA),fluorescence real time PCR (Roche,USA), Elispot plate reader (Bio-Sys,Germany). SigmaPlot 5.0 (SPSS,USA)

2.2 Study subjects

Ten antiretroviral treatment（ART） naïve HIV-1 recombinant subtype B/C infectors with infected time in 1 year, 25 ART-naive infectors with infected time more than 3 years and 10 HIV-1-seronegative healthy individuals were enrolled from Xinjiang, China. All individuals in this study were infected by HIV-1 B/C recombinant, as determined by gag, nef, and pol sequencing. Relevant clinical and demographic data of the study subjects are summarized in Table 1. There was significant correlation between the number of CD4 T cell and plasma viral load (p=0.00230 r2=0.251). The study was approved by the respective institutional review boards and all subjects gave written informed consent.

TABLE 1. Clinical and demographical information about the study subjectsa

Infected time	Statistics	Age (year)	Plasma viral load (RNA copies/ml) a	CD4 count (Per mm3) a	CD8 count (Per mm3) a
<1year	Mean	30	374083	467	1406
	Median	30	274900	475	1359
	Range	21～38	<LDL～1300000	252～715	730～2464
>3year	Mean	32	308127	324	1060
	Median	32	10510	321	967
	Range	17～44	<LDL～2415000	86～603	381～2619

a Measured at time of HIV-1-specific T-cell analysis, there were six infectors whose Plasma viral load less than lowest detection level.

2.3 Determination of CD4 cell count

The CD4 cell count from EDTA anticoagulated whole blood was performed by using FITC-conjugated CD3 antibody, PE-conjugated CD4 antibody, PC5-conjugated CD45 antibody and a Beckman-Coulter elite flow cytometry equipped with argon ion laser (488 nm) according to the manufacturer's instructions.

2.4 Determination of HIV-1 viral load

Plasma viral loads were detected by fluorescence real time PCR according to the manufacturer's instruction, the detection limit of PG assay is 100 HIV-1 RNA copies per ml.

2.5 Preparation of peripheral blood mononuclear cell

Peripheral blood mononuclear cells (PBMCs) were prepared from whole blood by density-gradient centrifugation on Ficoll-Hypaque. After washing twice with Hank's solution, the pellet was resuspended in R10 medium (RPMI 1640 that contained 10% fetal bovine serum, 100 U/ml penicillin, 100 μg /ml streptomycin, and 2 mmol L-glutamine/L) and the final concentration of PBMC was adjusted to 1.0×106 cells/ml.

2.6 Design of peptide matrix

Peptides consisted of a total of 123 overlapping peptides that spanned the HIV-1 genes encoding the Gag. All 15-mers overlapped by 11 amino acids. The sequences of the peptides were based on the HIV-1 clade B consensus sequence. All peptides were included in one-peptide matrix systems which included 23 peptide pools, respectively, at two different axes. Each peptide was represented in two different peptide pools, allowing for the identification of the respective peptide by responses in the two corresponding pools. This is exemplified for the identification of the GAG7937 peptide in Table 2 (e.g., Pool Y6 =peptides GAG7932 to GAG7943, aggregately 12 peptides; Pool X6= GAG7877, GAG7889, GAG7901, GAG7913, GAG7925, GAG7937, GAG7949, GAG7961, GAG7973 and GAG7985, aggregately 10 peptides). The final concentration of each peptide within a peptide pool was 50 μg/ml.

TABLE 2. Example of peptide matrix setup for Gag

	X1	X2	X3	X4	X5	**X6**	X7	X8	X9	X10	X11	X12
Y1	GAG7872	GAG7873	GAG7874	GAG7875	GAG7876	**GAG7877**	GAG7878	GAG7879	GAG7880	GAG7881	GAG7882	GAG7883
Y2	GAG7884	GAG7885	GAG7886	GAG7887	GAG7888	**GAG7889**	GAG7890	GAG7891	GAG7892	GAG7893	GAG7894	GAG7895
Y3	GAG7896	GAG7897	GAG7898	GAG7899	GAG7900	**GAG7901**	GAG7902	GAG7903	GAG7904	GAG7905	GAG7906	GAG7907
Y4	GAG7908	GAG7909	GAG7910	GAG7911	GAG7912	**GAG7913**	GAG7914	GAG7915	GAG7916	GAG7917	GAG7918	GAG7919
Y5	GAG7920	GAG7921	GAG7922	GAG7923	GAG7924	**GAG7925**	GAG7926	GAG7927	GAG7928	GAG7929	GAG7930	GAG7931
Y6	**GAG7932**	**GAG7933**	**GAG7934**	**GAG7935**	**GAG7936**	**GAG7937**	**GAG7938**	**GAG7939**	**GAG7940**	**GAG7941**	**GAG7942**	**GAG7943**
Y7	GAG7944	GAG7945	GAG7946	GAG7947	GAG7948	**GAG7949**	GAG7950	GAG7951	GAG7952	GAG7953	GAG7954	GAG7955
Y8	GAG7956	GAG7957	GAG7958	GAG7959	GAG7960	**GAG7961**	GAG7962	GAG7963	GAG7964	GAG7965	GAG7966	GAG7967
Y9	GAG7968	GAG7969	GAG7970	GAG7971	GAG7972	**GAG7973**	GAG7974	GAG7975	GAG7976	GAG7977	GAG7978	GAG7979
Y10	GAG7980	GAG7981	GAG7982	GAG7983	GAG7984	**GAG7985**	GAG7986	GAG7987	GAG7988	GAG7989	GAG7990	GAG7991
Y11	GAG7992	GAG7993	GAG7994									

a Example, shown in bold: a positive response to peptide Gag7937 would be reflected in positive responses in pools X6 and Y6

2.7 Characterization of HIV-1 specific T cell responses by Elispot assay

HIV-1 specific T lymphocyte responses were quantified by Elispot assay. Elispot assay was performed according to the manual of Human IFN-γ ELISPOT kit. Briefly, fresh PBMC were plated onto 96-well plates that had been precoated with 0.5 g of anti-IFN- γ monoclonal antibody; PBMC were added at a concentration of 100 000 cells per well in a volume of 100 μl of R10 medium (RPMI 1640, 10% fetal calf serum, 10 mmol/L HEPES buffer) with antibiotics (50 U of penicillin-streptomycin/ml). The final concentration of the peptides in the well was 5 μg/ml. Plates were incubated overnight at 37°C, 5% CO2, and developed. Wells containing PBMC and R10 medium were used as negative controls. Wells containing PBMC and phorbol 12-myristate 13-acetate (PMA) and inomysin served as positive controls. Duplicate experimental wells and quadruple control wells were used. The numbers of spots per well were counted using an automated Elispot plate reader, and the number of

specific T cells was calculated by subtracting the negative control values. A response was considered positive when the mean spot forming cell for the experimental wells was at least three times the mean SFC for the negative control wells and the mean SFC/106 cells in the experimental wells had to be more than 50 SFC/106 PBMC.

2.8 Statistical analysis

Statistical analysis and graphical presentation were done by SigmaPlot 5.0. Results are given as mean ± standard deviation (SD) or medians with ranges. Mann-Whitney Rank Sum test was used to test for significant differences between the magnitude of response of infectors at different stages, Spearman rank correlations were used to assess the relationships between responses and viral load or CD4 count.

3 Results

3.1 Gag specific T lymphocyte responses of Chinese HIV-1 recombinant subtype B/C infectors at different stages

Using the above-described peptide matrix approach, we screened a total of 35 HIV-1-infected individuals and 10 HIV-1-seronegative healthy individuals for Gag specific T lymphocyte responses in order to comprehensively assess the total breadth and magnitude of virus-specific responses on the single peptide level. None of HIV-1-seronegative healthy individuals was detected response. 25 (71.43%) subtype B/C HIV-1-infected individuals responded to one or more of the 123 peptides used in this study. The mean of magnitude of response was 2913 SFC/106 PBMC (median: 1762, range: 81~14032). The mean of breadth of response was 19.2 peptides/infector (median: 12, range: 2~71). Figure 1 shows the distribution of the number of positive peptide across the Gag for the infectors at different stages. There were 8 (80%) infectors with infected time in 1 year responsed. five most frequently recognized peptides were found. GAG7912 and GAG7951 lie in p24; GAG7895 lie in p17; GAG7979 lie in p7 and GAG7992 lie in p6 (Figure 1A). There were 17 (68%) infectors with infected time more than 3 years responsed. seven most frequently recognized peptides were found. GAG7896 lie in p17; six peptides: GAG7911, GAG7912, GAG7917, GAG7923, GAG7924 and GAG7945 lie in p24 (Figure 1B). The amino acid sequence, position, recognized rate and the mean, median, range of magnitude of response was showed in Table 3.

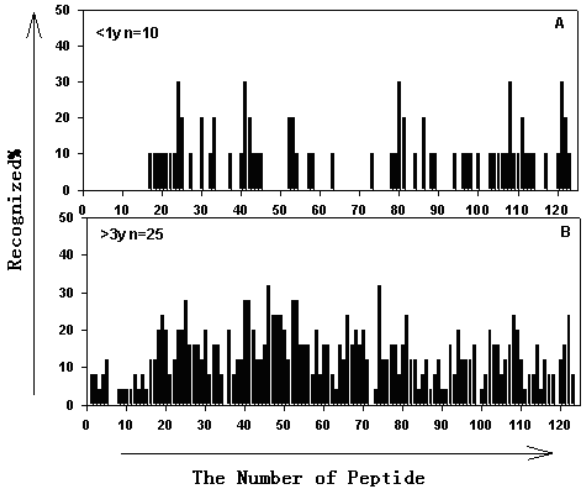

FIG. 1. Peptide recognition across the Gag of infectors at different stages. The 123 individual overlapping peptides are represented on the x-axis, and the corresponding percentage of study subjects with a response to the individual peptide are represented on the y-axis. The horizontal bar indicates the corresponding regions for the individual peptides.

TABLE 3. Most frequently recognized peptides in HIV-1 B/C recombinants infectors at different stages

Peptide	Amino acid position	Sequence	Recognized%		Magnitude(SFC/106PBMC) Mean(Median:Min~Max最大值)	
			<1year	>3year	<1year	>3year
GAG7895	Gag 93-107 = p17 93 -> 107	EVKDTKEALEKIEEE	30		340（345:210～465）	
GAG7896	Gag 97-111 = p17 97 -> 111	TKEALEKIEEEQNKS		28		139（61:45～572）
GAG7911	Gag 157-171 = p24 25 -> 39	KVVEEKAFSPEVIPM		28		164（78:33～626）
GAG7912	Gag 161-175 = p24 29 -> 43	EKAFSPEVIPMFSAL	30	28	373（373:343～403）	162（83:47～632）
GAG7917	Gag 181-195 = p24 49 -> 63	PQDLNTMLNTVGGHQ		32		166（109:15～623）
GAG7923	Gag 205-219 = p24 73 -> 87	INEEAAEWDRLHPVH		28		176（133:50～405）
GAG7924	Gag 209-223 = p24 77 -> 91	AAEWDRLHPVHAGPI		28		179（113:60～387）
GAG7945	Gag 293-307 = p24 161 -> 175	FRDYVDRFYKTLRAE		32		68（42:18～207）
GAG7951	Gag 317-331= p24 185 -> 199	MTETLLVQNANPDCK	30		253（306:61～391）	
GAG7979	Gag 429-443 = p7 52 -> p1 11	RQANFLGKIWPSHKG	30		348（375:134～537）	
GAG7992	Gag 481-495= p6 33 -> 47	KELYPLASLRSLFGN	30		248（266:63～415）	

3.2 The Difference between Gag specific T lymphocyte responses of Infectors at different Stages

For infectors with infected time in 1 year，the mean of magnitude of response was 2846 SFC/106 PBMC (median:3231, range:273~5627), the mean of breadth of response was 9.6 peptides/infector (median:9 , range:5~18); For infectors with infected time more than 3 years，the mean of magnitude of response was 2944 SFC/106 PBMC (median:1471, range:81~14032), the mean of breadth of response was 23.7 peptides/infector (median:16 , range:2~71); The magnitude of response of infectors with infected time in 1 year was obviously higher (P =0.021) than that of infectors with infected time more than 3 years. There was no difference (P =0.076) between breadths of these two group infectors.

3.3 The relationship between HIV-1-specific T-cell responses and plasma viremia or the number of CD4 count of Infectors at different Stages

For infectors with infected time in 1 year，there was no significant correlation between magnitude (P=0.885，r2 =0.002) or breadth (P=0.460，r2 =0.086)of specific T lymphocyte responses and plasma viral loads. No significant correlation between magnitude (P =0.160，r2 =0.275) or breadth (P =0.120，r2 =0.315)of specific T lymphocyte responses and CD4 counts; For infectors with infected time more than 3 years，there was a significant correlation between magnitude (Fig. 3. P=0.0318，r2 =0.269) of specific T lymphocyte responses and plasma viral loads, but no significant correlation between breadth (P =0.981，r2 =0.000) and response No significant correlation between magnitude (P =0.173，r2 =0.118) or breadth (P =0.974，r2 =0.000)of responses and CD4 counts.

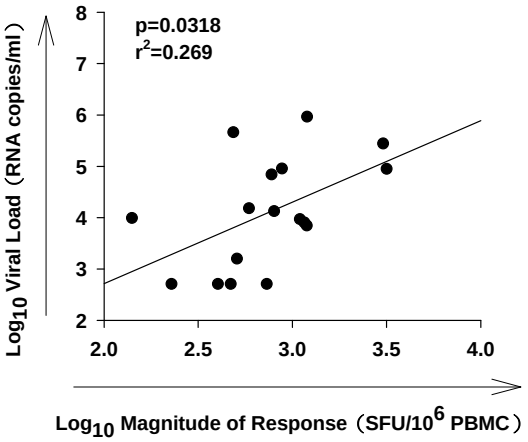

FIG. 2. Correlations between log10 magnitude of Gag specific T lymphocyte response and log10 plasma viral loads of with infected time more than 3 years

4 Conclusion

Gag encodes an important 55kD structure protein of HIV which could be dissociated to p17, p24, p7 and p6. Our results showed that infectors with infected time in 1 year responded five most frequently recognized peptides: GAG7912 and GAG7951 lie in p24; GAG7895 lie in p17; GAG7979 lie in p7 and GAG7992 lie in p6; infectors with infected time more than 3 years responded seven most frequently recognized peptides were found. GAG7896 lie in p17; six peptides: GAG7911, GAG7912, GAG7917, GAG7923, GAG7924 and GAG7945 lie in p24. In these peptides, GAG7911 and GAG7912, GAG7923 and GAG7924 are adjacent to each other. These two adjacent peptide might contain one epitope because the peptides we used were all 15-mers overlapped by 11 amino acids and the length of the epitope could be presented by MHC-I molecular is 8~11 amino acid. Our results also showed that the infectors recognized different region of Gag at different stages. This might be caused of variation of virus under immune pressure. It suggests that the mainly recognized region should be paying more attention for vaccine design and ore research on infectors of early stages should be conducted.

Our results showed that the magnitude of response of infectors with infected time in 1 year was obviously higher than that of infectors with infected time more than 3 years. Past research has found that the functional profile of HIV-specific CD8 T cells in progressors was limited. Their cell functions, such as degranulation, IFN-gamma, MIP-1beta, TNF-alpha, and IL-2 were impaired compared to that of nonprogressors [3].These functions could be restored partially by cultured with IL-2. Recent study also shown that the expression of granzyme B and interferon-γ of CD8+ T cells in HIV infection is dissociated[4]. The difference between magnitudes of response of infectors at different stages might coursed by decreased CD4 T cells or impaired functions of CD8 T lymphocytes.

The emergence and preservation of specific T lymphocyte are fundamental in the host defense against HIV-1 infection [5, 6]. But it remains controversial on the correlation between viral load and HIV-1 specific T cell responses, different studies of HIV-1 infected individuals have shown that there is an positive [7], negative[8] or no correlation[9]. This might be partly due to the different stages of infection or which parameters were measured. Our study showed a significant correlation between the magnitude of specific T lymphocyte response to HIV-1 Gag and the viral load infectors with infected time more than 3 years. These observations support the hypothesis that IFN-γ production of HIV-1-specific T lymphocyte is not main mechanism of control of viral replication and these effector cell expansions and contractions are driven by changes in antigen load [10]. The functional impairment of CD8 T lymphocyte responses in late-stage infection could not be reflected by gamma interferon-based screening techniques[11]. Future studies, such as the investigation of mechanism of control of viral replication and the “quality” of HIV-1-specific T cell responses[12, 13] are therefore needed to identify the correlates of immune mediated control of HIV-1 replication.

References

1. Ndongala ML, Peretz Y, Boulet S, Doroudchi M, Yassine-Diab B, Boulassel MR, et al. HIV Gag p24 specific responses secreting IFN-gamma and/or IL-2 in treatment-naive individuals in acute infection early disease (AIED) are associated with low viral load. Clin Immunol 2009.
2. Serwanga J, Shafer LA, Pimego E, Auma B, Watera C, Rowland S, et al. Host HLA B*allele-associated multi-clade Gag T-cell recognition correlates with slow HIV-1 disease progression in antiretroviral therapy-naive Ugandans. PLoS ONE 2009,4:e4188.
3. Oxenius A, Price DA, Easterbrook PJ, O'Callaghan CA, Kelleher AD, Whelan JA, et al. Early highly active antiretroviral therapy for acute

HIV-1 infection preserves immune function of CD8+ and CD4+ T lymphocytes. Proc Natl Acad Sci U S A 2000,97:3382-3387.
4. Kleen TO, Asaad R, Landry SJ, Boehm BO, Tary-Lehmann M. Tc1 effector diversity shows dissociated expression of granzyme B and interferon-gamma in HIV infection. Aids 2004,18:383-392.
5. Ueno T, Motozono C, Dohki S, Mwimanzi P, Rauch S, Fackler OT, et al. CTL-mediated selective pressure influences dynamic evolution and pathogenic functions of HIV-1 Nef. J Immunol 2008,180:1107-1116.
6. Wang YE, Li B, Carlson JM, Streeck H, Gladden AD, Goodman R, et al. Protective HLA Class I Alleles Restricting Acute-Phase CD8+ T Cell Responses are Associated with Viral Escape Mutations Located in Highly Conserved Regions of HIV-1. J Virol 2008.
7. Northfield JW, Loo CP, Barbour JD, Spotts G, Hecht FM, Klenerman P, et al. Human immunodeficiency virus type 1 (HIV-1)-specific CD8+ T(EMRA) cells in early infection are linked to control of HIV-1 viremia and predict the subsequent viral load set point. J Virol 2007,81:5759-5765.
8. Novitsky V, Gilbert P, Peter T, McLane MF, Gaolekwe S, Rybak N, et al. Association between virus-specific T-cell responses and plasma viral load in human immunodeficiency virus type 1 subtype C infection. J Virol 2003,77:882-890.
9. Bartovska Z, Beran O, Rozsypal H, Holub M. HIV-1-specific CD8(+) T cells do not correlate with viral load in HIV-1-positive patients. Acta Virol 2007,51:59-61.
10. Alter G, Tsoukas CM, Rouleau D, Cote P, Routy JP, Sekaly RP, Bernard NF. Assessment of longitudinal changes in HIV-specific effector activity in subjects undergoing untreated primary HIV infection. Aids 2004,18:1979-1989.
11. Draenert R, Verrill CL, Tang Y, Allen TM, Wurcel AG, Boczanowski M, et al. Persistent recognition of autologous virus by high-avidity CD8 T cells in chronic, progressive human immunodeficiency virus type 1 infection. J Virol 2004,78:630-641.
12. Addo MM, Draenert R, Rathod A, Verrill CL, Davis BT, Gandhi RT, et al. Fully differentiated HIV-1 specific CD8+ T effector cells are more frequently detectable in controlled than in progressive HIV-1 infection. PLoS ONE 2007,2:e321.
13. Jiang Y, Karita E, Castor D, Jolly PE. Characterization of CD8+ T lymphocytes in chronic HIV-1 subtype A infection in Rwandan women. Cell Mol Biol (Noisy-le-grand) 2005,51 Suppl:OL737-743.

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