

The prevalence and immunological features of anti-glomerular basement membrane antibody in patients with HIV/AIDS

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Wenjing Wang
Capital Medical University

Xiao-yu Jia Jia
Peking University First Hospital

Zhao Cui
Peking University First Hospital

Yan Chen
Capital Medical University

Wei Wang
Capital Medical University

Jin-li Lou
Beijing youan hospital

Ming-hui Zhao
Peking University First Hospital

Sun Ying

 ying.sun@ccmu.edu.cn *Corresponding Author*

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Abstract

Background

Anti-glomerular basement membrane disease (GBM) is an autoimmune disease caused by the deposition of circulating anti-GBM antibodies. The main target antigens are the non-collagen region of the $\alpha 3(\text{IV})\text{NC1}$ ($\alpha 3$ chain of type IV collagen). EA and EB are two classical antigen epitopes on $\alpha 3$. It has been reported that anti-glomerular basement membrane (GBM) antibodies could be detected in HIV/AIDS patients, but its immunological characteristics are still unclear. In this study, the immunological characteristics of the target antigens were clarified.

Methods

Total 93 HIV/AIDS patients and 20 healthy volunteers were selected in Beijing Youan Hospital from 2017 to 2018. Recombinant human $\alpha 1$ - $\alpha 5(\text{IV})\text{NC1}$, chimeric protein E A , E B were used as solid phase antigens. Enzyme-linked immunosorbent assay was employed to measure concentrations and subtypes of serum IgG autoantibodies specially against GBM.

Results

Five out of the 93 patients with HIV/AIDS had low to moderate levels of anti-GBM antibodies. However, these patients presented with no clinical manifestation of any kidney injury or pulmonary hemorrhages. Compared with HIV/AIDS patients with negative antibodies, there were no significant differences in gender, age, CD4 + T cell count and HIV viral load. All five patients recognized non-collagenous domain1 (NC1) of alpha 3 chain of type IV collagen [$\alpha 3(\text{IV})\text{NC1}$] as classic anti-GBM patients, followed by $\alpha 5(\text{IV})\text{NC1}$. The antibodies against $\alpha 3(\text{IV})\text{NC1}$ was IgG3 predominant, but did not react with either of the classic epitopes on $\alpha 3$ (E A and E B).

Conclusion

These data suggest a distinct immunological profile of anti-GBM antibodies in patients with HIV/AIDS, and might explain the non-pathogenic features of HIV/AIDS associated anti-GBM antibodies.

Background

The acquired immunodeficiency syndrome (AIDS), originally discovered in the early 1980s, is an infectious disease caused by the human immunodeficiency virus (HIV). It can cause acquired

immunodeficiency in humans, leading to various infections and malignant tumors. Patients with HIV/AIDS often suffer from multiple organ dysfunction and death. Among them, 30% have abnormal renal function, while HIV/AIDS-related kidney disease has become the third leading cause of end-stage renal disease in HIV/AIDS patients[1]. Kidney disease in HIV-infected subjects manifests in a variety of ways, including acute kidney injury (AKI), HIV-associated kidney disease, comorbid chronic kidney disease (CKD) and treatment-related kidney toxicity[2]. HIV-associated nephropathy (HIVAN) is one of the most common recognized HIV-associated kidney problems[2-4].

A number of autoantibodies, including anti-nuclear (ANA), anti-neutrophil cytoplasmic (ANCA) and anti-glomerular basement membrane (GBM) antibodies can be detected in HIV-infected individuals[5]. Anti-GBM antibodies are pathogenic of anti-GBM disease which often manifests as the aggressive crescentic glomerulonephritis[2]. These antibodies primarily target the non-collagenous domain1 (NC1) of alpha 3 chain of type IV collagen ($\alpha 3(\text{IV})\text{NC1}$) which normally expresses in the glomerular and alveolar basement membranes[3]. However, in most of the cases with anti-GBM antibodies detected in HIV/AIDS patients, there were no obvious clinical manifestation of renal damage[1, 5, 6]. It is still in controversy whether these antibodies are pathogenic as the ones in classic anti-GBM patients. In the present study, therefore, we measured the anti-GBM antibodies in the serum of HIV/AIDS patients and analyzed, for the first time to our best knowledge, the immunological characteristics of these antibodies in order to provide some clues.

Methods

Subjects and sera

Ninety-three patients with HIV/AIDS, diagnosed in Beijing Youan Hospital from January to December in 2018, were enrolled in the present study. According to the diagnostic criteria, all patients were diagnosed as the asymptomatic stage or AIDS since blood samples from patients with acute infection are difficult to collect. Sera of positive control were obtained from patients with classical anti-GBM disease diagnosed in Peking University First Hospital. Normal human sera were obtained from 20 healthy volunteers from Beijing Youan Hospital.

All serum samples were collected and stored at $-80\text{ }^{\circ}\text{C}$ until use. Clinical data of HIV/AIDS patients

were collected at the time of diagnosis and during follow-up. The present study was in compliance of the Declaration of Helsinki and approved by the ethics committee of Beijing Youan Hospital and Peking University First Hospital. Informed consent was obtained from each individual.

Preparation of recombinant human α (IV) NC1 and chimeric proteins

The recombinant human α (IV)NC1 and chimeric proteins were prepared as described previously.[7] Briefly, the cDNAs of the α 1- α 5 NC1 domain of type IV collagen was ligated into the X-type collagen triple helix guide sequence and cloned into the pcDNA plasmid. The constructed plasmid was stably transfected into human embryonic kidney 293 cell line and harvested from the culture solution. And the recombinant protein was purified using published procedures[7].

The construct EA consists entirely of the α 1(IV)NC1 domain, containing 45 amino acids of α 3(IV)NC1 E_A region. The construct E_B is completely composed of α 1(IV)NC1 that contain 37 amino acids of α 3(IV)NC1 E_B region. Construction of non-E_{AB} was constructed in the context of α 3(IV)NC1, in which the EA and EB regions were replaced by the corresponding amino acids of α 1(IV)NC1[7, 8].

Detection of anti-GBM antibodies in serum samples using enzyme-linked immunosorbent assay

A home-based enzyme-linked immunosorbent assay (ELISA) was performed to measure anti-GBM antibodies in the sera. In brief, a mixture of the recombinant human α 1- α 5(IV)NC1 proteins was diluted with carbonate buffer solution (CBS 0.05 M, pH 9.6) to the concentration of 1.0 μ g/mL for each antigen, and coated onto half of the wells of the polystyrene microtiter plate (Nunc, Roskilde, Denmark). The other half of the wells were coated with bovine serum albumin (BSA) at the same concentration in 0.05 M CBS as the antigen-free wells to exclude non-specific binding. Incubation was carried out at 37°C for 60 min. After washing, the serum samples (1:100 diluted with a dilution buffer containing 0.64 M NaCl, 0.008 M Na₂HPO₄·12H₂O, 0.003 M KCl, 0.002 M KH₂PO₄, 0.1% Tween-20, pH 7.4) were then added to the antigen coated and antigen-free wells respectively. Incubation resumed at 37°C for 60 min. After washing, alkaline phosphatase-conjugated goat anti-human IgG (Fc specific, Sigma, USA, 1:6000) was added, and incubation resumed at 37°C for 60 min. After washing, 50 μ L of substrate solution (containing 1 mg/mL p-nitrophenyl phosphate, 1.0 M diethanolamine, 0.5 M MgCl₂,

pH 9.8, Sigma, USA) was added into the wells for color development which was measured spectrophotometrically at 405 nm (Bio-Rad, Tokyo, Japan) 15min later. The net absorbance values were OD values of the antigen-coated wells minus the OD values of the antigen-free wells. Each serum sample was tested in duplication, while the samples were re-tested when the standard deviation between the wells >10%.

Detection of antigen and epitope specificity in sera of HIV/AIDS patients with anti-GBM antibodies

To detect antigen and epitope specificity, plate wells were respectively pre-coated with each of the five recombinant human α (IV)NC1 or the chimeric proteins containing E_A, E_B and non-E_{AB} (1 μ g/mL per protein). Serum samples of HIV/AIDS patients with anti-GBM antibodies were diluted at 1:50 and added in duplication into the wells at 37°C for 60 min. After washing, alkaline phosphatase-conjugated goat anti-human IgG (Fc specific, Sigma, USA) was added, and incubation resumed at 37°C for 60 min. Color was developed and measured as described above. Serum sample from healthy volunteers were used to build up the cutoff values using mean+2 SD for each α chain and the chimeric protein.

Detection of distribution of IgG subclass against α 3(IV)NC1 in HIV/AIDS patients with anti-GBM antibodies

The detection of IgG subclasses against α 3(IV)NC1 was performed as previously reported[7]. In brief, the recombinant human α 3(IV)NC1 proteins were diluted at 1 μ g/mL and coated onto half of the wells of a polystyrene microtiter plate. The other half of the wells were coated with BSA as antigen-free wells. Diluted serum samples at 1:100 were then added to the antigen and BSA coated wells respectively, and incubated at 37°C for 60 min. After washing, horseradish peroxidase labeled mouse monoclonal antibodies against human IgG1, IgG2, IgG3, and IgG4 (Fc specific, Southern Biotech, USA, 1:2000) were added at 37°C for 60 min. After washing, color was developed by adding 50 μ L of substrate (Tetramethyl benzidine, TMB, Invitrogen, USA) into the wells for 15 min. Reaction was terminated by adding stop solution and color measured at 450 nm.

Statistics

Continuous variables were expressed as means \pm SD or medians. Comparison between continuous

variables was conducted by t test for normally distributing data or nonparametric test for non-normally distributing data. Differences between qualitative data were analyzed using χ^2 or Fisher exact test. All statistical analyses were two-tailed and P value <0.05 was considered significant.

Analysis was performed using the IBM SPSS statistics 24.0.

Results

The Demographic and Clinical data of patients with HIV/AIDS

A consecutive of 93 patients diagnosed with HIV/AIDS from January to December in 2018 was enrolled in the present study, including 73 males and 20 females with a mean age of 36 years. Among these subjects, 48/93 (51.6%) patients were asymptomatic while the remaining were at the stage of AIDS. 32 (34.4%) of all the patients had a history of infections, including pulmonary, blood or other tissues, and 14 of them had laboratory evidence. All patients received anti-viral therapies and were followed up for a median time of 6 months. Two (2/93, 2.15%) patients died from pulmonary infection and multi-organ failure during their hospitalization, while 3 (3/93, 3.23%) were lost at the end of follow-up. The demographic and clinical data are shown in Table 1.

The prevalence of circulating anti-GBM antibodies in patients with HIV/AIDS

A total of 5 sera (5.37%) from the 93 patients showed reactivity against a mixture of all five human α (IV)NC1 chains. Among them, 4 were male and one female, with a mean age of 39.8 ± 11.3 years. None of these 5 patients with anti-GBM antibodies had hemoptysis or any kind of kidney injuries including proteinuria or hematuria. However, these 5 patients presented significantly higher levels of serum urea (5.3 ± 0.6 vs. 4.2 ± 1.2 mmol/L, $p = 0.027$), creatinine (78.5 ± 25.1 vs. 59.7 ± 15.8 μ mol/L, $p = 0.014$) and lower levels of estimated glomerular filtration rate (eGFR, 103.2 ± 24.1 vs. 121.7 ± 19.9 ml/min/1.73m², $p = 0.048$), compared with the AIDS/HIV patients without the antibody. In HIV/AIDS patients without anti-GBM antibodies, urinalysis indicated proteinuria (mostly 1+) in 16 cases (18.8%, 16/88) and hematuria (mostly 1+) in 3 cases (3.41%, 3/88), respectively.

There were no significant differences in HIV viral load and CD4⁺ T lymphocyte count between HIV/AIDS patients with or without anti-GBM antibodies (Table 2). However, the prevalence of infections in patients with anti-GBM antibodies seemed to be higher. Three out of the 5 patients (60%,

3/5) possessing anti-GBM antibodies had bacterial infections, compared to the corresponding figure of 11/88 (12.5%) in anti-GBM negative patients ($P = 0.004$).

Antigens and epitope specificity of anti-GBM antibodies in the sera of HIV/AIDS patients

The recombinant human $\alpha 1$ to $\alpha 5(IV)NC1$ were used to determine antigen specificity of anti-GBM antibodies. Among these 5 patients, one failed to react with either of the five α chains. Sera of the remaining four patients (4/4, 100%) all recognized $\alpha 3(IV)NC1$, while 2 sera (2/2, 50%) recognized $\alpha 5(IV)NC1$. Only one patient (1/5, 20%) reacted with $\alpha 1(IV)NC1$, $\alpha 2(IV)NC1$, $\alpha 4(IV)NC1$ (Table 3). Sera from positive controls recognized both E_A and E_B . However, none of the three chimeric proteins could be recognized by the sera from the 4 HIV/AIDS patients with anti- $\alpha 3(IV)NC1$ antibodies (Table 3).

IgG subclass distribution of serum antibodies against $\alpha 3(IV)NC1$

IgG subclasses were further detected in the 4 HIV/AIDS patients with anti- $\alpha 3(IV)NC1$ antibodies. Among them, IgG3 was the dominant subtype (100%, 4/4), while IgG1 and IgG4 were detected in 1/4 case, respectively. IgG2 subclass was not detected in any sera (Table 4).

Follow up of HIV/AIDS patients with anti-GBM antibodies

All the 5 patients possessing anti-GBM antibodies were followed up for 6–12 months (Table 5). Among them, 2 patients showed slight decline of eGFR (decreased by 10.4% and 11.4% respectively). One patient, whose serum recognized all five α chains, developed elevated level of urinary protein (semi-quantitative detection at 1.0g/L). The remaining 3 patients showed no renal impairment.

Discussion

Kidney dysfunction caused by HIV infection is related to a number of reasons including the course of disease, viral load status, drug treatments, co-infections, tumorigenesis and metabolic disorders[9, 10]. There have been a series of cases reported in HIV/AIDS patients possessing autoantibodies against GBM, complicating the dissection of the causes for kidney involvement[5]. Although classic anti-GBM antibodies are recognized to be pathogenic, most HIV/AIDS cases with anti-GBM antibodies manifested no features of anti-GBM nephritis[1, 5]. In order to further elucidate the pathogenesis of these antibodies, we investigated, for the first time to our best knowledge, the immunological characteristics of the anti-GBM antibodies in HIV/AIDS patients and their associations with clinical

data.

A total of 5 out of 93 HIV/AIDS patients were found to possess low to moderate levels of anti-GBM antibodies. However, no kidney injury or hemoptysis was found in the 5 patients at the time of testing. Therefore, these antibodies in HIV/AIDS patients seem to be non-pathogenic, as consistent with previous reports[1, 6]. It has been reported that the production of anti-GBM antibody is related to CD4⁺ T cell count,¹ in which the authors found a significant correlation between the presence of anti-GBM antibodies and CD4 counts less than 400/L[5]. In the present study, one of the 5 anti-GBM positive patients, who had the antibodies against all 5 α chains, also had a high viral load, suggesting that the CD4⁺ T cell count might associate with HIV viral load in the point of view of antigenic reorganization. However, we did not find significant difference in CD4⁺ T cell count and HIV viral load between HIV/AIDS patients with or without the antibodies, probably because all patients in our study had taken antiviral drugs. Furthermore, our data revealed that the prevalence of anti-GBM antibodies were associated with bacterial infections. Previous studies have shown that *P. carinii* alveolar injury or the host response to the organism might affect the Goodpasture antigen or a similar antigen, because Goodpasture's syndrome could be triggered by an alveolar lesion induced by *P. carinii* pneumonia[1, 11]. Anti-GBM disease associated with other infections including dengue virus had also been reported[12]. Taken together, these suggest that there might be an important link between infection and the emergency of anti-GBM antibodies, which needs to be further investigated.

Although the production of autoantibodies associated with HIV infections are thought to be caused by poly-clonal activation of CD4⁺ T cells, we found that anti-GBM antibodies in HIV/AIDS patients are rather specific and recognized a relatively narrower antigen spectrum. Almost all sera recognized α 3(IV)NC1 and half of them reacted with α 5(IV)NC1, while the recognition of other α chains were limited. It has been shown that the main target antigen recognized by GBM antibody is α 3(IV)NC1, and that the level of α 3(IV)NC1 antibody is a key factor in the extent of kidney damage in classic anti-GBM diseases.[13, 14] Recently, antibodies against α 5(IV)NC1 has been proved to be pathogenic in Goodpasture's disease as well[15]. However, in the present study, none of these HIV/AIDS patients

with anti-GBM antibodies had clinical evidence of renal injuries. It was of notice that most of the anti-GBM antibodies in these patients were borderline positive, with only one patient possessing moderately elevated level of antibodies against $\alpha 3(\text{IV})\text{NC1}$. Moreover, none of these sera recognized the two major epitopes on $\alpha 3(\text{IV})\text{NC1}$, namely E_A and E_B . It has been reported that patients with high levels of circulating antibodies against the specific epitopes E_A and E_B have a more severe renal disease at diagnosis as well as a worse prognosis[16]. Data derived from animal models suggest a non-pathogenic but augmenting effect of anti-EB antibodies in the development of crescentic glomerulonephritis[17]. Some other studies have demonstrated that in human anti-GBM disease the levels of anti- E_B antibodies are positively associated with severity of renal damage[16]. Therefore, we speculate that the antibodies in these patients might recognize non-pathogenic epitopes on $\alpha 3$ and $\alpha 5(\text{IV})\text{NC1}$, therefore exerting no kidney injuries.

Savige et al. found that plasma from 18 of 105 HIV infected individuals were positive for anti-GBM antibodies[18]. Most of the patients also have borderline levels of anti-GBM antibodies, though the prevalence is much higher than the present study. The authors suggested that these antibodies may arise from polyclonal activation, or be due to “sticky” serum[18], especially when sera diluted at 1:8. In the present study, all serum samples were diluted at 1:100 to reduce false positivity, which might be one of the reasons for a lower prevalence of anti-GBM antibodies in our study.

Conclusions

Five out of 93 HIV/AIDS patients were found to have low to moderate levels of anti-GBM antibodies. However, none of the patients manifested any kind of kidney injuries or pulmonary hemorrhages. These antibodies recognized a specific and narrow antigen spectrum encoding $\alpha 3$ and $\alpha 5(\text{IV})\text{NC1}$, but had a distinct epitope repertoire. The differences in immunological characteristics may explain the non-pathogenic features of HIV/AIDS associated anti-GBM antibodies.

Declarations

Ethics approval and consent to participate

Ethical approval for the study was granted by the Ethics Committee of Beijing You’an hospital. As a result of the retrospective design of the study and the corresponding policy, the local ethics

committee confirmed that the conditions for exemption from informed consent were met and that participants were not required to give informed consent.

Consent for publication

Not applicable.

Availability of data and materials

Our database contains sensitive data, which can provide clinical data and personnel information about our patients and lead to the identification of these patients. Therefore, according to the restrictions and provisions of the relevant organizations, these data can not be publicly provided. However, if the requirements are reasonable, datasets used and/or analyzed in this study can be obtained from the authors.

Competing interests

All of the authors read and approved the final manuscript. All of the authors declare that they have no competing interests.

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Authors' contributions

XYJ and ZC were responsible for the study design. WJW, JLL were responsible for data collection, specimen collection and experiments; WJW, XYJ and ZC were analyzed and interpreted the data; WJW, XYJ drafted the manuscript; WJW, XYJ, ZC, YC, WW, JLL, MHZ and SY critically revised the manuscript. All authors read and approved the final manuscript.

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Author details

1 Department of Immunology, School of Basic Medical Sciences, Capital Medical University, Beijing 100069 China.

2 Beijing Youan Hospital, Capital Medical University, Beijing 100069, China.

3 Renal Division, Peking University First Hospital; Institute of Nephrology, Peking University; Key Laboratory of Renal Disease, Ministry of Health of China; Key Laboratory of CKD Prevention and Treatment, Ministry of Education of China; Beijing 100034, China

4 Peking-Tsinghua Center for Life Sciences, Beijing 100871, China

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Tables

Table 1. The Demographic and Clinical data of patients with HIV/AIDS

Parameters	n=93
Age (years)	36.1±10.7
Gender (male/female)	73/20
Stage (Asymptomatic/AIDS)	45/48
CD4 ⁺ T cell count (/mL) (median, range)	292 (0-1734)
HIV load (cope/ml) (median, range)	0 (0-3257132)
bacterial infections (n, %)	14/73, 19.2%
Hemoglobin (g/L)	126.6±30.8
Serum urea (mmol/L)	4.2±1.2
Serum creatinine (μmol/L)	60.8±16.7
eGFR (ml/min/1.73m ²)	120.8±20.3
Proteinuria (n, %)	16, 17.2%

Table 2. Comparison of clinical features of HIV/AIDS patients with and without anti-GBM antibodies

	with anti-GBM antibodies (n=5)	without anti-GBM antibodies (n=88)	P value
Age (years)	36.6±9.8	36.3±11.0	0.939
Gender (male/female)	4/1	69/19	0.933
Stage (Asymptomatic/AIDS)	4/1	44/44	0.192
CD4 ⁺ T cell count (/mL)	520.6±171.6	146.8±214.0	0.308
HIV Load (Detected/NOT)	1/4	29/59	0.052
Hemoglobin (g/L)	141.0±32.1	125.8±30.7	0.284
Serum urea (mmol/L)	5.3±0.6	4.2±1.2	0.027
Serum creatinine (μmol/L)	78.5±25.1	59.8±15.7	0.014
eGFR (ml/min/1.73m ²)	103.2±24.1	121.8±19.8	0.046
Bacterial Infections (n, %)	3, 6%	11, 12.5%	0.004
Proteinuria	0	16, 18.2%	0.295

Table 3. Target antigens of circulating anti-GBM antibodies in HIV/AIDS patients

	Antigen distribution					Epitope on α3(IV)NC1		
	α1(IV)NC1	α2(IV)NC1	α3(IV)NC1	α4(IV)NC1	α5(IV)NC1	E _A	E _B	Non-E _{AB}
P1	0.050	-0.051	-0.024	-0.04	-0.043	-	-	-
P2	0.155 ^Δ	0.612 ^Δ	0.324 ^Δ	0.079 ^Δ	0.095 ^Δ	0.026	-0.016	0.028
P3	0.039	0.031	0.142 ^Δ	-0.086	0.131 ^Δ	-0.006	0.009	0.011
P4	0.001	0.015	0.138 ^Δ	-0.001	0.024	0.005	0.066	0.151
P5	0.031	-0.003	0.132 ^Δ	0.005	0.011	0.043	0.037	0.067
cut-off value	0.085	0.178	0.066	0.067	0.066	0.150	0.156	0.205

Δ Positive OD value

Table 4. IgG subclass distribution of circulating antibodies against α3(IV)NC1 in HIV/AIDS patients

	IgG subclass distribution				
	IgG1	IgG2	IgG3	IgG4	
P1	-	-	-	-	
P2	-0.07	-0.002	0.196[△]	0.004	
P3	0.078	0.005	0.138[△]	-0.015	
P4	0.133	-0.003	0.252[△]	0.281[△]	
P5	0.225[△]	0.007	0.136[△]	-0.137	
Cutoff value	0.163	0.031	0.048	0.098	
cut-off value	0.085	0.178	0.066	0.067	

△ Positive OD value

Table 5. Clinical data of anti-GBM-positive HIV/AIDS patients during follow-up

No	Gender	Stage	on the time of testing						follow-up after one year						
			CD4	Viral load	UPRO	UREA	CRE	eGFR	CD4	Viral load	UPRO	UREA	CRE	eGFR	
P1	Male	AIDS stage	42	TND	-	6.4	109.3	69.1		58	TND	-	4.2	84.8	86.9
P2	Male	Asymptomatic	407	791856	-	5.4	74.0	124.5		451	42	2+	5.0	84.5	111.6
P3	Male	Asymptomatic	440	<40	-	4.9	67.1	119.1		460	TND	-	6.2	73.1	114.2
P4	Male	Asymptomatic	776	TND	-	5.2	96.5	86.7		608	TND	-	5.2	106.9	76.8
P5	Female	Asymptomatic	459	TND	-	5.0	45.4	116.7		645	TND	-	5.6	51.9	110.8

Notes and Units: CD4: / μ L; Viral load: copy/ml; UPRO: Urine Protein; UREA: serum urea (mmol/l); CRE: serum creatinine (μ mol/L); eGFR: estimated glomerular filtration rate (ml/min/1.73m²); TND: target not detected.