NEUTRALIZATION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1) WITH ANTIBODY FROM CARRIERS' PLASMA AGAINST HIV-1 PROTEIN P17

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Summary. – It was investigated whether human antibody against HIV-1 protein p17 (anti-p17) in HIV carriers' plasma has the ability to neutralize the infectivity of HIV. By the pretreatment of HIV-1 with anti-p17 from HIV carriers, progeny HIV-1 production from cells infected with virus pretreated with anti-p17 was suppressed and/or delayed. The neutralizing activity of anti-p17 was decreased in the presence of recombinant p17. The latter obviously masked the neutralizing activity of anti-p17. The relevant epitope(s) on p17 is located apparently on the surface of HIV virions and the binding of anti-p17 to p17 impairs the infectivity of HIV. This implies that anti-p17, if stably present in HIV carriers' plasma, may also play an important role in reducing the infectivity of HIV-1 *in vivo*.

Key words: human immunodeficiency virus type 1; p17; human anti-p17 antibody; neutralization; matrix protein; vaccination

Introduction

Two virus-neutralizing sites located within HIV p17 region (Glu^{12}-Ile^{19} and Ala^{100}-Glu^{105}) have been determined. Binding of monoclonal antibodies (MoAbs) with neutralizing ability was investigated using a series of sequential overlapping hexapeptides of HIV p17 protein (Papsidero *et al.*, 1989; McNicholl and McDougal, 1994).

Originally, the anti-HIV activity of the antiserum against thymic hormone, thymosin α_1, was found due to a region of homology between thymosin α_1 and p17. Forty four to 50% homology was found between the regions of amino acids (aa) 11-28 on thymosin α_1 and aa 92-109 on the gag protein (Sarin *et al.*, 1986; Naylor *et al.*, 1987). On this basis, an 30 aa-long synthetic peptide analogue, HGP-30, which corresponds to the region Tyr^{86}-Ala^{115} in p17 region, has become one of the HIV vaccine candidates, and entered the phase 1 of clinical trial (Kahn *et al.*, 1992).

Appearance of epitope-specific antibodies, e.g. anti-HGP-30, reflected the clinical status of the HIV-1-infected subjects under study. In fact, anti-HGP-30 declined to non-detectable levels as patients progressed to ARC/AIDS (Jiang *et al.*, 1992). It has also been reported that HIV carriers

Abbreviations: aa = amino acid; AIDS = acquired immunodeficiency syndrome; anti-p17 = anti-p17 antibody; ARC = AIDS-related complex; ELISA = enzyme-linked immunosorbent assay; gp120 = 120 K glycoprotein of HIV; HIV-1 = human immunodeficiency virus type 1; MoAb = monoclonal antibody; p17 = 17 K protein of HIV; p24 = 24 K protein of HIV; PBMC = mononuclear cells from peripheral blood; PHA = phytohaemagglutinin; PHA-PBMC = PBMC activated with PHA; rgp120 = recombinant gp120; rp17 = recombinant p17

with high titer of anti-p17 may not be at risk for the earlier onset of AIDS as compared to those without detectable anti-p17 (Kageyama and Kurimura, 1990; Choudhury *et al.*, 1992; Lange *et al.*, 1987).

p17 antigen is expressed, at least in part, on the surface of HIV-infected cells. Several MoAbs against to p17 reacted with live cell infected with HIV-1 and HIV-2 (Ikuta *et al.*, 1989; Shang *et al.*, 1991). These results led us to speculate that HIV may be the target neutralizable with anti-p17.

Therefore, it has been postulated that anti-p17 binds to an epitope on the surface of HIV virion and neutralizes it. In this study, the binding of anti-p17 HIV and the possible antiviral activity of anti-p17 in patients' plasma were investigated.

Materials and Methods

Cells. Mononuclear cells from peripheral blood (PBMC) of HIV-sero-negative donors' were prepared by density-gradient-centrifugation using 5.7% Ficoll 400 (Pharmacia) and 9% sodium-isotalamate (Daiichi Pharmaceutical, Tokyo, Japan), activated with phytohaemagglutinin (PHA, Difco) for 3 days, and used as target cells in acute infection system. Cells of HTLV-1-transformed T cell line MT-4 (Miyoshi *et al.*, 1982) were used to assess the infectivity of HIV-1_{LAI} treated with human anti-p17 purified by affinity coulumn chromatography. CCRF-CEM cells (Foley *et al.*, 1965), originating from a patient with acute lymphocytic leukemia, was used for the analysis of anti-p17 binding.

Affinity column chromatography of anti-p17. Of several human plasma specimens, five with high titer of anti-p17 were selected (data not shown). These specimens were subjected to affinity column chromatography. Recombinant p17 (rp17) (Saito *et al.*, 1992) coupled to CNBr-activated Sepharose 4B (Pharmacia) was used as the ligand to trap the anti-p17 from plasma. Antibody eluted by 0.1 mol/l glycine-HCl pH 7.2 was adjusted to neutral pH and dialyzed for the subsequent HIV-neutralization assay.

Enzyme-linked immunosorbent assay (ELISA). Anti-p17 purified by affinity column chromatography was subjected to ELISA to confirm its binding affinity to p17. The latter and recombinant gp120 (rgp120) (Intracel Corp., Cambridge, USA) were used to capture antibodies in sandwich ELISA. Briefly, 25 µl of anti-p17 purified by affinity column chromatography was mixed with equal volume of rp17 (0.1 mg/ml) (Saito *et al.*, 1992), rgp120 (0.1 mg/ml, Intracel Corp.,) or PBS (control) and incubated at room temperature for 1 hr. Each pretreated antigen-antibody mixture was exposed to rp17 (100 ng/well) and rgp120 (50 ng/well) fixed on a 96-well plate, and incubated for 15 hrs. Biotinylated anti-human IgG was used as the second antibody. Peroxidase-avidin, o-phenylene diamine (2.6 mg/ml) and hydrogen peroxide (0.03%) were also used in ELISA.

Neutralization assay. Purified human anti-p17 (40 µg in 100 µl) was preincubated with rp17 (20 µg in 100 µl) at 37°C for 1 hr and exposed to 150 $TCID_{50}$ of HIV-1_{LAI} at 37°C for 1 hr. MT-4 cells were incubated with this antibody-HIV mixture at 37°C for 1 hr (infection), washed twice, and suspended in complete medium (RPMI 1640 supplemented with 10% foetal calf serum) at the concentration of 10^5 cells/ml. On day 3 after infection, HIV production in the culture supernatant was assessed by the measurement of p24 antigen by capture ELISA (Abbott, Abbott Park, USA).

In the subsequent assay, other four human anti-p17 preparations (0.05 µg in 20 µl), also purified by affinity column chromatography, were mixed with 20 or 200 $TCID_{50}$ in equal volume of freshly isolated HIV-1_{JH3} (Komiyama *et al.*, 1989) and incubated at 37°C for 1 hr. PBMC activated with PHA (PHA-PBMC) for 2 days were infected with this antibody-HIV mixture. Cells were extensively washed and cultured for 7 days. HIV production in the culture supernatant on day 7 after infection was assayed by ELISA (Abbott).

Flow cytometry analysis and electron microscopy. CCRF-CEM cells infected with HIV-1_{LAI} and cultured for 9 days were incubated with mouse MoAb anti-p17, HyHIV-15 (Liu *et al.*, 1995) or C415 (recognition site at aa 87-115 in gag region), or anti-gp120 0.5 ß, kindly provided by Dr. Matsushita, Kumamoto University (Matsushita *et al.*, 1988) at 37°C for 30 mins under gentle shaking. The cells were susbsequently incubated with fluorescein isothiocyanate- (Dako, Glostrup, Denmark) or colloid gold (diameter 15 nm, Biocell Research Ltd, Cardiff, UK) -conjugated anti-mouse IgG as the second antibody. Each sample was then fixed with 1% formalin or 2.5% glutaraldehyde and subjected to flow cytometry analysis with Lysys Application Program on Consort 30 computer system (Becton-Dickinson), or to electron microscopy (Katsumoto *et al.*, 1990), respectively. Excessive antibody concentrations were used throughout the assay to saturate all the epitopes.

Results

Binding specificity of human anti-p17 from sero-positive individuals

In ELISA, human anti-p17 10-104-1 was strongly bound to rp17 (mean A_{450} was 0.395). This binding was blocked by rp17 (mean A_{450} decreased to 0.041). On the other hand, the binding affinity of this antibody to rgp120 was negligible (mean A_{450} was 0.009). These results indicated that the reac-

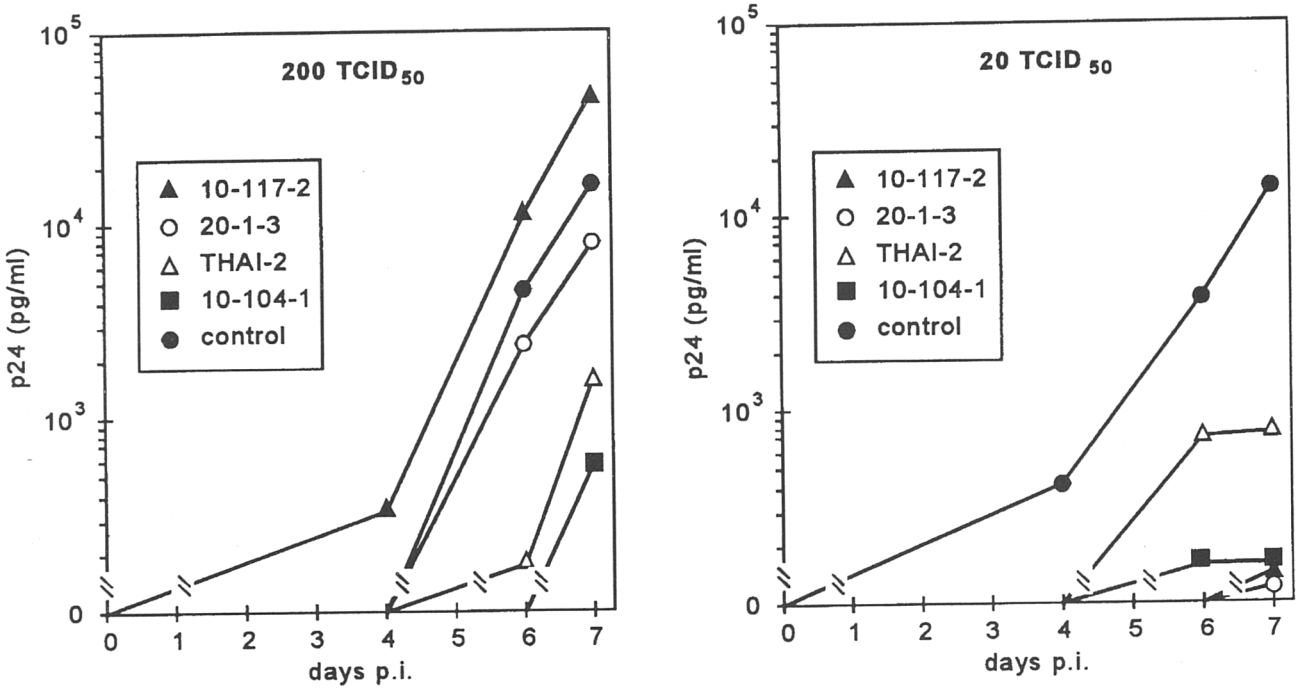

Fig. 1
Neutralizing activity of human anti-p17 prepared by affinity chromatography
2 x 10^6 PHA-PBMC were infected with 200 or 20 $TCID_{50}$ of HIV-1_{JH3} preincubated with human anti-p17, and cultivated for 7 days. Then HIV (p24) production in the culture supernatant was assessed. Anti-p17 specimens: 10-117-2, 20-1-3, Thai-2, 10-104-1.

Table 1. Reduced neutralizing effect of purified human anti-p17 on HIV by its binding to rp17

	HIV production (the amount of p24 in ng/ml) after exposing the cells to the following mixtures		
	HIV[c]	HIV+anti-p17[b]	HIV+anti-p17+rp17[a]
Duplicate values	712, 846	386, 314	672, 628
Geometric mean	776	348	650
Suppression (%)	0	55	16

[a]40 μg of affinity-purified anti-p17 was mixed with 20 μg of rp17 and incubated with 250 $TCID_{50}$ of HIV in 100 μl. This mixture was used as inoculum for the infection of 10^6 MT-4 cells. On day 3 after infection, production of progeny virus in the culture supernatant was assessed.
[b]rp17 omitted.
[c]Both anti-p17 and rp17 omitted.

tivity of anti-p17 purified by affinity chromatography was specific for p17 but not for gp120.

Neutralizing activity of human anti-p17

The infectivity of HIV was significantly reduced by the binding of anti-p17 (Table 1). When infecting HIV was pre-treated with anti-p17, a lesser amount of progeny virus was detected in the culture medium (348 ng/ml) as compared to the control (776 ng/ml). This neutralizing effect of anti-p17 was confirmed by an experiment, in which anti-p17 was mixed with rp17 and incubated with the infecting HIV. Consequently, the latter could not bind the antibody and was able to infect cells. Eventually, the level of progeny virus production increased to 650 ng/ml of p24.

In the PBMC culture system, the production of progeny virus was much more clearly suppressed (Fig. 1). When HIV (200 $TCID_{50}$) was preincubated with two different human anti-p17 (THAI-2, 10-104-1) and inoculated onto PHA-PBMC, virus production was apparently delayed and suppressed as compared to the control (Fig. 1). The suppressive effect was more expressed, when the antibodies were applied on a smaller virus inoculum. When 10-fold-diluted HIV (20 $TCID_{50}$ per inoculum) was used for the infection, the level of p24 production on day 7 after infection was significantly suppressed in all the cases (Fig. 1).

p17 on the surface of HIV-1-infected cells

As compared to the fluorescence intensity of HIV-infected-CCRF-CEM cells exposed only to anti-mouse IgG con-

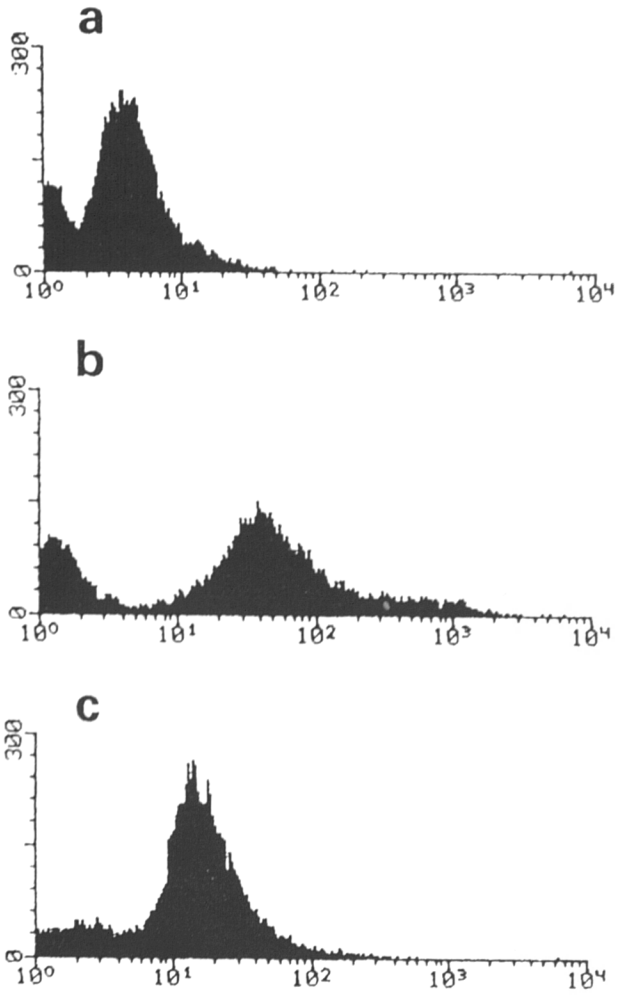

Fig. 2
Flow cytometry analysis. The profile of staining of HIV-infected CEM cells with anti-p17 C415 and anti-gp120 0.5β
FITC-conjugated anti-mouse IgG was used as the second antibody to stain HIV-infected CEM cells with bound MoAbs. (a) Control cells. No staining was performed with either anti-p17 or anti-gp120. Cells were incubated only with FITC-conjugated anti-mouse IgG. (b) Staining with anti-p17. (c) Staining with anti-gp120. Abscissa: number of cells. Ordinate: relative intensity.

jugated with FITC (Fig. 2a), these cells were more clearly stained with both the anti-p17 (Fig. 2b) and anti-gp120 (Fig. 2c) MoAbs and their staining pattern obtained by flow cytometry was different. Whereas almost all the cells were stained with anti-gp120, just a few cells were positive with anti-p17 (Fig. 2).

p17 on the surface of HIV virions

While there was no non-specific binding of anti-mouse IgG conjugated with colloidal gold beads to the surface of HIV particles (Fig. 3a), this antibody bound to anti-gp120 (Fig. 3b) or anti-p17 (Fig. 3c) was clearly observed on the surface of budding virions and those released from or close to the infected cells.

Discussion

In the prevention of the onset of AIDS, the antibodies against HIV play obviously certain role in fighting HIV. The role of anti-gp120 has already been discussed and anti-gp120 is accepted as neutralizing antibody. However, the variability of amino acid sequence of gp120 region, which may generated by mutations, will allow the emergence of strains escaping from the immuno-surveillance by anti-gp120 (Graham and Wright, 1995). Another neutralizing antibody, anti-p17 (Sarin *et al.*, 1986; Naylor *et al.*, 1987), has an advantage in a less frequent mutation of p17 as compared to gp120 (McNicholl and McDougal, 1994; Henderson *et al.*, 1992). In the natural course of HIV infection, anti-p17 may bind to HIV and soon be taken up. In fact, anti-p17 disappeared from the plasma in the early stage of HIV infection before the onset of AIDS, while anti-gp120 remained continuously present (Weiss, 1993). This makes us expect that if anti-p17 would be stably present in the plasma, it could decrease the titer of HIV. Therefore the investigation of the role of anti-p17 should be further continued.

In this study, we focused our attention on the question whether the infectivity of HIV could be significantly impaired by a treatment with anti-p17 derived form human plasma, and whether anti-p17 could bind to HIV virions as can be observed on HIV-infected cells. Our experiments showed that when HIV was preincubated with the affinity-purified human anti-p17, the progeny virus was not efficiently produced as assessed by p24 level in the culture medium. This anti-HIV activity was diminished in the presence of recombinant p17; it clearly indicated that anti-p17 itself contributed to the reduction of HIV infectivity. However, it is still unclear how anti-p17 can effectively block the HIV replication. Recently, it was reported that p17 acts as the nuclear localization signal and stabilizes genomic RNA and/or cDNA in the cytoplasm of non-dividing cells, and also in the case of cell-to-cell infection (Bukrinsky *et al.*, 1993; von Schwedler *et al.*, 1994; Karageorgos *et al.*, 1993). These findings indicate that p17 imported into cytoplasm from adsorbed HIV seems to be indispensable for the replication especially in growth-arrested cells. However, those findings are insufficient to explain the impairment of HIV infectivity by anti-p17 in dividing cells such as PHA-activated PBMC employed in this study. If the anti-HIV activity of anti-p17 does not affect the transport of genomic RNA in the cells, the binding of this antibody to HIV may interfere with the adsorption and penetration of this virus.

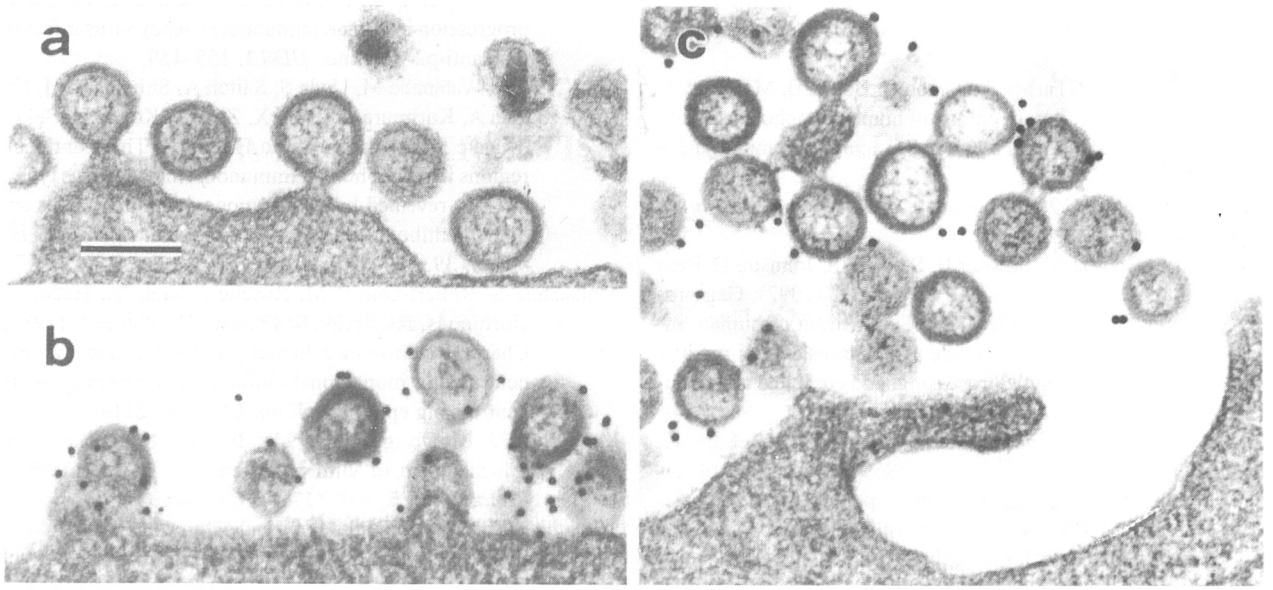

Fig. 3
Recognition of HIV surface molecules with colloidal gold-labelled antibodies by electron microscopy
(a) No treatment with primary antibody (control). (b) Mouse anti-gp120 MoAb 0.5β was used for the detection of gp120 molecules. (c) Mouse anti-p17 MoAb HyHIV-15 was bound to p17 on the surface of HIV. Bar = 200 nm. Magnification 70,000 x.

We demonstrated also the presence of molecules on the surface of HIV particles which can bind anti-p17. An epitope of p17 on HIV was clearly recognized with a mouse MoAb against p17. Interestingly, the epitope of p17 was not seen on the plasma membrane, but on HIV particle. However, it is still unclear how gag (p55) and gag-pol (p160) polyproteins assemble underneath the viral envelope. The conformation of these proteins in the viral membrane remains to be explained too. It seems that immature viruses bud immediately after gag and gag-pol proteins anchor their N-termini in the inside of viral envelope. Not a few viral particles which do not package gag and gag-pol proteins may also bud immediately after the envelope spikes are prepared on the cell-membrane to be used to compose the viral membrane. This may be one of the reasons why anti-p17 was mainly bound to HIV but not to HIV-infected cells, and why viral envelope can much more easily be recognized than gag protein among HIV-infected cells. In fact, the number of p17-positive cells was observed to be lesser than that of gp 120-positive ones even at the peak of antigen-detectable phase in the acute infection system.

The amino acid sequence recognized with MoAb used in this study is mostly corresponding to that of HGP-30 already reported as a vaccine candidate against HIV infection (Kahn *et al.*, 1992). Undoubtedly, one of the major problems of vaccine development is the variability of the envelope sequence among HIV strains. In this context, p17 has an advantage of a target molecule in attempting to reduce the HIV replication. Thus, p17 is expected to be a useful target especially to prevent HIV strains resistant to anti-envelope antibody from the replication. Anti-p17 may act synergistically with anti-gp120 to reduce the HIV load. When used against mutated HIV that excaped from immuno-surveillance by anti-gp120, anti-p17 is supposed to reduce the HIV load, and *vise versa.*

In this study, it is speculated that the neutralizing activity of human anti-p17 resides in binding to p17 molecule on the surface of HIV. Further studies on p17 will contribute to the elucidation of the regulation of HIV replication.

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