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Antigenic determinants synthesized in a library of randomly cloned fragments of the HIV-1 genome

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A DNA expression library of randomly selected fragments of the HIV-1 genome was constructed and used to search for antigenic determinants. A large segment of the HIV-1 provirus was sonicated, and 150–250 bp DNA fragments were cloned in a system of expression vectors developed to obtain high yields of recombinant proteins in *Escherichia coli*. The expressed library was immunoscreened with sera of AIDS patients. Eleven identified immunoreactive clones were found to correspond to already known and new antigenic regions of HIV-1 proteins gp41, p24, and reverse transcriptase.

Human immunodeficiency virus-1; Immunoepitope; Expression; Acquired immunodeficiency syndrome; Diagnostics

1. INTRODUCTION

Immunoreactive fragments of HIV envelope and internal proteins are now widely used for AIDS diagnostics and development of vaccine candidates [1–5]. Many antigenic determinants of HIV proteins were identified and expressed in various systems with the use of recombinant DNA techniques [3–5]. At the same time, all attempts to obtain an effective anti-HIV vaccine candidate based on known antigenic determinants have been so far unsuccessful. It seems thus desirable to screen the whole HIV genome with the aim of revealing new potentially useful antigenic determinants.

We developed a simple and quick method for immunoscreening the viral genome based on cloning random fragments of viral DNA and their expression in *Escherichia coli*. An analysis of about 2000 expressed clones permitted us to reveal two dominant immunogenic regions (belonging to p24 and gp41), and also three minor epitopes (two in reverse transcriptase, and one in gp41).

2. MATERIALS AND METHODS

2.1. *DNA library of randomly cloned fragments of the HIV-1 genome*

To create the library, pSP64 plasmids containing the *Sst*I-*Sst*I fragment of HIV-1 provirus (pBH10 isolate [6]) were sonicated and a fraction of 150–250 bp long DNA fragments was isolated. After treatment of the fragments with DNA polymerase I and subsequent ligation to an 8-mer oligonucleotide *Bam*HI-linker (Boehringer), they were cloned into the *Bam*HI site of the pVAM2 vector (see below). An *E. coli* C600 strain was used as a bacterial host for the clone library.

2.2. *Analysis of randomly cloned DNA*

Double stranded fragments of viral DNA cloned in the pVAM2 vector were sequenced essentially as described [7]. CGAAGAGCATCCCAAGATCGCA primer corresponding to the C-terminus of IFN-γ was used. The nucleotide sequences were analyzed using the DNASTAR programs and data bank.

2.3. *The system of expression vectors*

The vectors for expression in three reading frames, pVAM1, pVAM2 and pVAM3 (fig.1) were constructed, each from a *Hin*dIII-*Hpa*I fragment of a run-away plasmid pBEU50 [8] (containing an *ori* and β-lactamase gene), a *Hin*dIII-*Pst*I insert of the same plasmid-containing human IFN-γ gene under control of pTrp*, a synthetic *Pst*I-*Hpa*I adaptor with *Eco*RI and *Bam*HI sites in one of three possible reading frames, and a universal transcription terminator. This set of vectors allows one to clone DNA fragments in three reading frames and to express fusion proteins composed of IFN-γ and a polypeptide of interest at the N- and C-terminus, respectively.

To test the vectors, a *Sau*3A-*Sau*3A fragment of *gag* encoding a. a. 78–437 of the p55 precursor (pGAG7 plasmid), and a *Bgl*II-*Bam*HI fragment of *env* encoding a. a. 466–752 of p160 (pENV38 plasmid) were cloned in pVAM2 and pVAM3, respectively. The clones were expressed in *E. coli* yielding recombinant immunoreactive proteins in amounts up to 10–15% of the total cell protein. They were further used as position control.

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Abbreviations: INF-γ, human γ-interferon; IAA, indole-3-acrylic acid; a. a., amino acid; pTrp, tryptophane promoter

* The pBEU50 plasmid with the INF-γ gene insert under the control of pTrp was constructed at the Laboratory of Human Gene Structure and Function, M.M. Shemyakin Institute of Bioorganic Chemistry, by Drs A.V. Chestukhin, K. Tikhonenko, S.A. Tsarev and S. Markova. A detailed description of the construction will be published elsewhere.

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2.4. *Immunological analysis of the library*

The transformed *E. coli* cells were grown on Petri dishes (Ap 50 mg/l, Trp 10 mg/l) for 18 h at 28°C, transferred to nitrocellulose filters and grown under the same conditions for 2 h. The filters were then put on Petri dishes with agarized M9 medium (50 mg/l Ap and 20 mg/l IAA) and grown for 4 h at 42°C.

After the cells were lysed by chloroform treatment for 10 min, the filters were dried and preincubated for 3 h in 3% BSA, 0.05 M Na-phosphate buffer (pH 6.8), 0.1% Tween 20. The pretreatment was followed by incubation with sera taken from AIDS patients and diluted with *E. coli* C600 cell lysates, addition of protein A-peroxidase conjugates and staining with 4-chlorine-1-naphtol.

Positive clones were additionally tested by ELISA on polystyrene plates. An overnight cell culture grown at 28°C in M9 (50 mg/l Ap, 10 mg/l Trp) was diluted 10-fold with the same medium but without Trp, grown for 4 h at 28°C, and then 4 h at 42°C in the presence of 20 mg/l IAA. The cells were precipitated, resuspended in 6 M guanidine chloride, diluted with sodium carbonate-bicarbonate buffer, incubated for 16 h at 4°C and used in ELISA.

3. RESULTS AND DISCUSSION

A library of more than 2000 clones harboring DNA fragments about 200 bp long of the HIV-1 provirus was immunoscreened. Fig.2 presents one of the stained nitrocellulose filters as an example. Clearly, the lysed cell clones are characterized by differential staining. Only heavily stained clones were found to be immunoreactive in ELISA tests on plates. The ELISA tests were repeated with various sera that increased the fidelity of detecting the immunoreactive clones and allowed us to compare the immunoreactivity of the cloned regions of viral proteins.

Eleven immunoreactive clones were revealed and

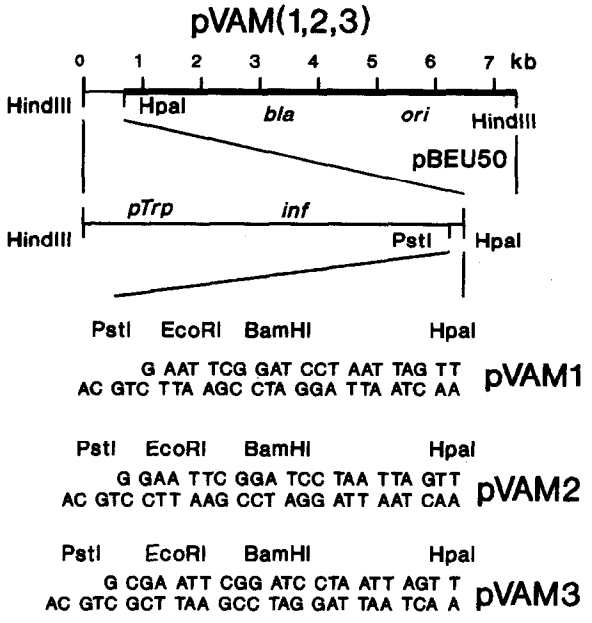

Fig.1. Structure of expression vectors pVAM. The bold line refers to DNA of the pBEU50 plasmid; the thin line represents the fragment containing the IFN-γ gene, pTrp, and a synthetic oligonucleotide. The nucleotide sequences of the vectors are given in the lower part of the figure.

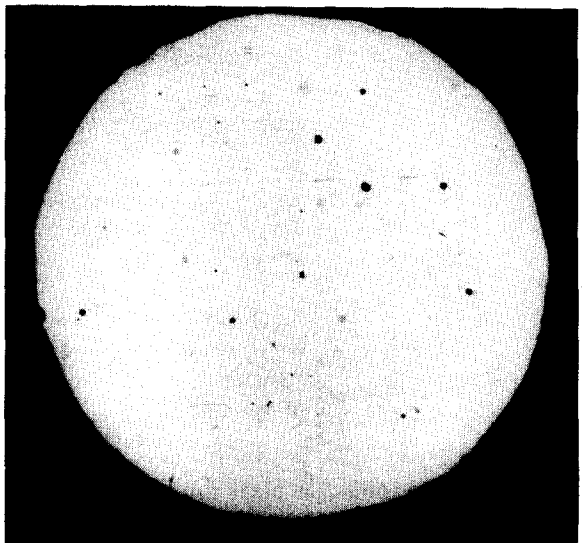

Fig.2. Immunostaining of expressed clones on a nitrocellulose filter.

characterized by this procedure (fig.3). Four clones (1–4) corresponded to a known gp41 immunodominant region (a. a. 586–609). Lysates of these clones were strongly immunoreactive with the majority of the sera used in ELISA tests. Nevertheless, there existed a number of sera that reacted selectively with clones 2–4 but not with clone 1. This result supports the data that

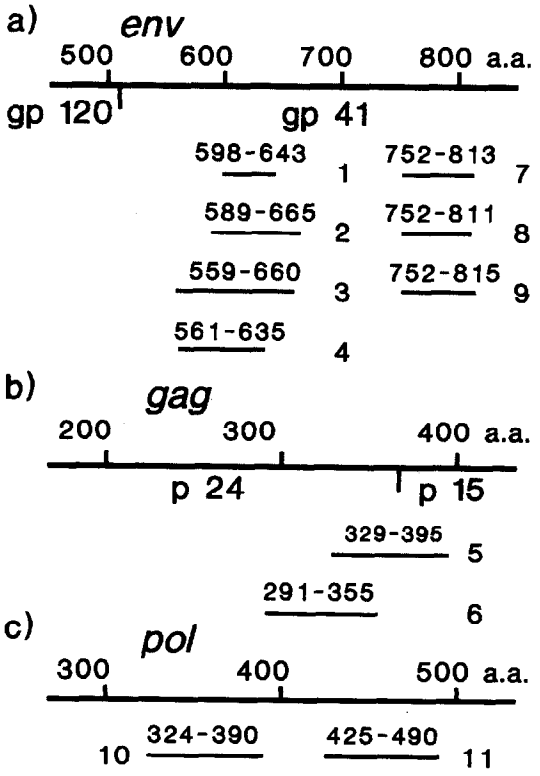

Fig.3. Identified immunogenic fragments of viral proteins encoded by the randomly cloned provirus DNA fragments. The a. a. are numbered in accordance with DNASTAR data bank for the BH10 isolate of HIV-1.

Table 1

The immunogenic regions of some HIV-1 proteins, published previously and identified in this work

Immunogenic region (a. a. number according to the DNASTAR data bank)				
gag (p55)	*pol*	*env* (gp120)	*env* (gp41)	
309–376 [9]*	324–390**	504–518 [6]	598–609 [4]	752–856 [7]
329–395**	425–490**	466–548 [7]	586–606 [5]	732–836 [8]
291–355**		405–523 [8]	598–643**	752–813**
			589–665**	752–811**
			559–660**	752–815**
			561–635**	

* predicted
** this work

the region of a. a. 589–598 plays an important role in the functioning of one of the gp41 epitopes [1,2]. We also detected another gp41 immunogenic region of a. a. 752–820 (clones 7–9). The fact that the gp41 fragments in all these clones begin with a. a. 752 is due to the location of the *Bam*HI site. In ELISA tests, clones 7–9 reacted significantly weaker than clones 1–4.

The expression of two clones (5 and 6) resulted in recombinant proteins containing p55 fragments which span the region of a. a. 291–390 and seem to include the main antigenic determinant of p24. However, there were a number of sera which reacted in ELISA tests with the lysate of clone 5 only but not of clone 6, suggesting the existence of more than one epitope in this region. Moreover, two fairly weak immunogenic regions were also detected in *pol* (reverse transcriptase, clones 10 and 11).

Table 1 summarizes immunogenic regions of HIV-1 proteins *gag*, *pol* and *env* both published earlier and found in this work. Our data on the immunogenicity of p24 and gp41 are in good agreement with the literature [1,2,4–6]. Besides, we have managed to find two rather weak new immunogenic regions in *pol*.

At the same time we would like to note that the previously described [3–5] immunogenic region close to the gp120 C-terminus was not spotted by screening the expression library. This could be explained either by the absence of appropriate antibodies in the sera used for screening or by underrepresentation of particular genomic fragments in the library, although the analysis performed is rather preliminary and not complete. The epitopes found by the analysis of expressed randomly cloned fragments of the HIV-1 provirus can be used separately or in various combinations in AIDS diagnostic kits and for construction of new vaccine candidates.

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REFERENCES

[1] Gnann, J.W., Nelson, J.A. and Oldstone, M.B.A. (1987) J. Virol. 61, 2639–2641.
[2] Wang, J.J.G., Steel, S., Wisniewolski, R. and Wang, C.Y. (1986) Proc. Natl. Acad. Sci. USA 83, 6159–6163.
[3] Palker, T.J., Matthews, H.J., Clark, M.E., Cianciolo, G.J., Randall, R.R., Langlois, A.J., White, G.C., Safai, B., Snyderman, R., Bolognesi, D.P. and Naynes, B.E. (1987) Proc. Natl. Acad. Sci. USA 84, 2479–2483.
[4] Windheuser, M.G. and Wood, C. (1988) Gene 64, 107–109.
[5] Samuel, K.P., Seth, A., Zweig, M., Showalter, S.D. and Papas, T.S. (1988) Gene 64, 121–134.
[6] Shaw, G.M., Hahn, B.H., Arya, S.K., Groopman, J.E., Gallo, R.C. and Wong-Staal, F. (1984) Science 226, 1165–1171.
[7] Chen, E.Y. and Seeburg, P.H. (1985) DNA 4, 65–170.
[8] Uhlin, B.E., Schweickart, V. and Clark, A.J. (1983) Gene 22, 255–265.
[9] Koito, A., Hattori, T., Matsushita, S., Maeda, Y., Nozaki, C., Sagawa, K. and Takatsuki, K. (1988) AIDS Res. Hum. Retrovir. 4, 409–417.