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Identification of a potent synthetic HIV1 immunogen compromising gag-P24 tandem T- and B-cell epitopes

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Recent studies indicate that the gag gene products may play a crucial role in the immune response against HIV infection since clinical progression to AIDS is associated with a reduction in the level of circulating antibodies to gag p24 and antibodies raised against p17 peptide can inhibit HIV1 infection in vitro. Using conventional structure prediction algorithms for T-cell and B-cell epitopes, we have selected and chemically synthesized several gag peptides. In particular, an unconjugated HIV1-p24 peptide containing both B- and T-cell epitopes in tandem plus Freund's adjuvant induced a strong antibody response in both mice and rabbits against p24 and its precursor p55 as judged by immunoblotting. In addition, the peptide presented in the appropriate MHC context was shown to be highly stimulatory for p24 specific murine T-cell clones.

Acquired immunodeficiency syndrome; Vaccine design; Antigenic epitope

1. INTRODUCTION

Recent studies have indicated that the gag gene products may play a crucial role in eliciting an immune response against HIV infection. The clinical progression of AIDS is associated with a reduction in circulatory antibodies to the gag p24 protein, and antibodies raised against a p17 peptide are capable of inhibiting HIV-1 in vitro [1,2]. Since the T-helper determinant (THD) of Hepatitis B (HB) core protein enhances anti-HB S antigen responses [3] we undertook to identify and study the possible gag-THD in HIV. Using conventional structure prediction algorithms for T-cell and B-cell epitopes [4–7], several gag peptide vaccine candidates were selected and synthesized (Table I).

This paper demonstrates that an unconjugated HIV1-p24 peptide (GPKEPFRDYVDRFYKTLRAEQASQEV), containing both predicted B- and T-cell epitopes in tandem is capable of inducing a strong antibody response in mice against p24 and its precursor p55. In addition, the peptide presented in the appropriate MHC context was shown to be highly stimulatory for p24-specific T-cell clones.

2. MATERIALS AND METHODS

2.1. *Peptides synthesis and purification*

Peptides were chemically synthesized using an Applied Biosystem 430-A Peptide Synthesizer, and cleaved from resin by HF. Peptides were purified by reverse-phase HPLC and their amino acid analyses were in good agreement with the theoretical compositions.

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2.2. *Immunogenicity of tandem T-B cell epitopes*

The immunogenicity of individual synthetic peptides (50 μg) was tested by immunizing Balb/c (H^{2d}) mice (Charles River) and rabbits (Maple Lane Farm, Ont., Canada) with peptides emulsified in complete Freund's adjuvant, followed by a booster injection 2–3 weeks later with the same peptides emulsified in incomplete Freund's adjuvant. Antisera raised against these peptides were collected 2 weeks after the last challenge and tested for their reactivity against HIV1 antigens by ELISA and immunoblotting.

2.3. *Assay for synthetic T-cell epitopes*

The immunogenicities of synthetic peptides were assessed by their ability to stimulate the proliferation of a long-term $L3T4^+$ T-cell line (HIV-1 p24/10) specific for a synthetic 104-mer peptide corresponding to the C-terminal half of p24 [8].

2.4. *ELISA*

Reactivities of the peptide-specific murine antisera against recombinant core proteins and antigens of HIV-1 were determined by using test kits purchased from Abbott Laboratories (IL, USA). Procedures for performing these tests were followed as described by the manufacturer, except for the following modifications. The polystyrene beads coated with recombinant material or anti-HIV antibody were treated with phosphate-buffered saline (pH 7.2) containing 5% (v/v) normal mouse serum for 2 h at room temperature before being used for the tests. In addition, a sheep anti-mouse IgG peroxidase conjugate (absorbed with human serum proteins, Sigma Chemical Co.) was used to detect the binding of specific mouse IgG antibodies to the recombinant core proteins or antigens of HIV-1.

2.5. *Immunoblots*

Antibodies raised in mice and rabbits against the synthetic peptides were tested for their immunospecificity using the immunoblot technique. HIV-1 viral proteins on strips were purchased from Bio-Rad and immunoblots were performed according to the manufacturer's specification. Anti-peptide sera were diluted 1:100 and 1:500 for mice and rabbit sera, respectively. Protein A alkaline phosphatase conjugate (Bio-Rad) was used as the reporting group. The substrate NBT:BCIP (Bio-Rad) was used for color development.

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Table I

Immunogenicity of the predicted T-B cell epitopes in the gag gene products of HIV-1

Peptides	Sequence[b]	Antibody response[a]	
		Mice	Rabbits
P17A	EELRSLYNTVAT	–	–
P17B	DTKEALDKIEEEQNKSKKKA	–	N.D.
P24A	RTLNAWVKVVEEKAFSPEVIP	–	+ + +
P24B	LKETINEEAAEWDRVHPVHAG	–	+ + +
P24C	GQLREPRGSDIAGTTSTLQEQI	–	–
P24D	IPVGEIYKRWIILGLNKIVRMYSP	–	+ +
P24E	GPKEPFRDYVDRFYK	–	–
HIV1-p24	GPKEPFRDYVDRFYKTLRAEQASQEV	+ + +	+ + +
P24F	LEEMMTACQGVGGPGHKARVLAEA	+ +	–
P24G	TETLLVQNANPDCKTILKALGPAA	+ +	+
P15A	ARNCRAPPKKGCWKCGKEGHQMKDC	–	N.D.[c]

[a] Antibody responses were assayed by immunoblot analysis. The negative sign represents antisera that do not recognize p24 and its precursor p55 in immunoblots; the positive sign indicates that the antisera recognize p24 and p55. The +, + +, + + + represent the dilution of the sera: 1:100, 1:500, and 1:2000, respectively

[b] Peptide sequences are based on the LAV isolate sequence reported by Alison et al. [15].

[c] N.D., not determined

3. RESULTS AND DISCUSSION

Several examples (diphtheria, tetanus and HB) have shown that protective antibodies against a specific disease can be elicited by the administration of specific components of the pathogen [9]. The envelope protein (gp160) of HIV-1 has been tested as a candidate recombinant or vaccinia expressed vaccine against HIV, and although it can induce virus-specific antibodies, it has failed to protect primates against challenge with wild-type HIV isolates. In addition, two regions of the protein gp160, residues 735–752 and 846–860, have been shown to suppress the normal lymphocyte proliferative response to mitogens [10]. Peptide-mediated immunosuppression may play an important role in the pathogenesis of the disease [11,12]. These results stress the need of a rational design for a synthetic vaccine against AIDS. To design the best candidate vaccine, strong viral B-cell neutralizing epitopes (BE) containing a high degree of conserved sequence between viral isolates must be linked to potent T-helper determinants (THD) to yield an intense and long-lasting cross-protective antibody response. It is, therefore, important to locate the most potent THD of the various HIV antigens, encoded by the env, gag, and pol genes. The number of linear THD and BE of the gp160 protein have been characterized [13]. Although the B- and T-epitopes of gag and pol proteins have been predicted by standard algorithms [14], these epitopes have yet to be determined experimentally.

Using conventional structure prediction algorithms for T-cell and B-cell epitopes [4–7], several gag peptides have been synthesized and purified by RP-HPLC (Table I). To assess the immunogenicity of the gag- peptides, rabbits and inbred Balb/c mice were injected with individual peptides (50–100 μg) emulsified in complete

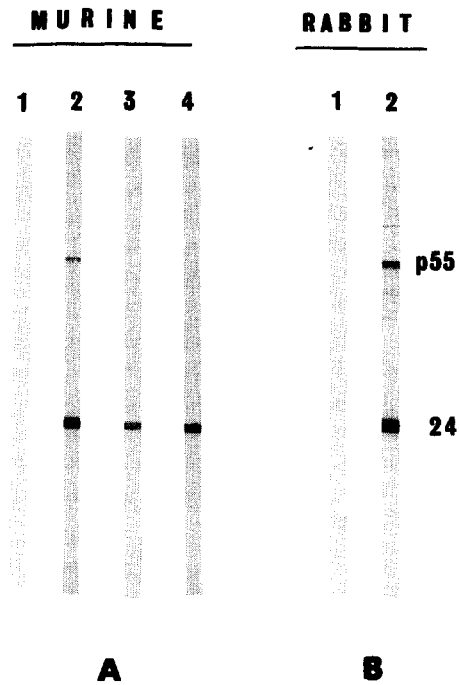

Fig. 1. Immunoblot of anti-HIV1-p24 antisera. Nitrocellulose immuno-strips of HIV-1 gene products (Bio-Rad) were reacted with murine and rabbit anti-HIV1-p24 antisera. A: lane 1, pooled sera from unprimed Balb/c mice; lane 2, pooled antisera from immunized Balb/c mice; lane 3, pooled antisera from C57BL/6 mice; lane 4, pooled antisera from C3H mice. B: lane 1, rabbit preimmune serum; lane 2, antiserum from a rabbit primed and boosted with HIV1-p24 peptide.

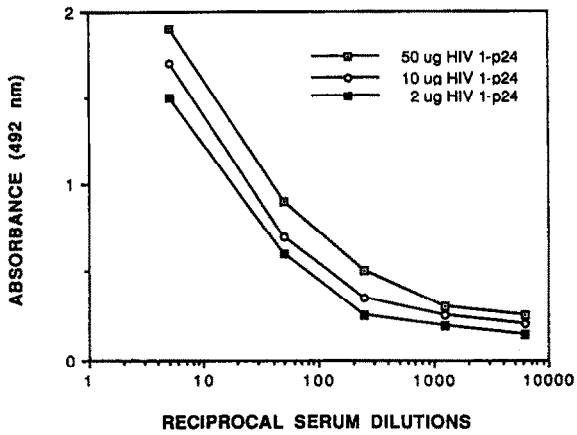

Fig. 2. Reactivities of HIV1-p24-specific Balb/c mice antisera against recombinant p24 as determined by ELISA.

Freund's adjuvant. After a booster dose with the same immunogen in incomplete Freund's adjuvant, sera were collected and tested for peptide-specific and gag-specific antibodies in immunoblots. The data summarized in Table I, indicate that not all gag peptides were capable of inducing a strong antibody response against p24 and its precursor p55 both in mice and rabbits, as judged by immunoblot analysis. In fact, peptide HIV1-p24 is the only one which consistently induced a strong antibody response against p24 in these animals (Fig.1A,B). These results confirm that peptide HIV1-p24 contains both B- and T-cell epitopes in tandem. Therefore, all further work has been concentrated on HIV1-p24.

In order to assess the potency of HIV1-p24, mice were immunized with increasing amounts of peptide, emulsified in incomplete Freund's adjuvant (2 to 50 μg). Mouse antisera generated with all three doses recognized gag-p24 and its precursor in immunoblot analysis (data not shown). The reactivity of mouse anti-HIV1-p24 antisera against gag-p24 was evaluated by ELISA. As shown in Fig. 2, all three antisera reacted well with recombinant p24 in a dose-dependent fashion. The immunogenicity of the synthetic peptide HIV1-p24 was further assessed by its ability to stimulate the proliferation of a long-term L3T4$^+$ T-cell line (HIV1-p24/10) specific for a synthetic 104-mer peptide corresponding to the C-terminal half of p24 [8]. The results summarized in Table II, showed that HIV1-p24 was capable of inducing a strong proliferative response in this T-cell line.

To determine whether the synthetic peptide HIV1-p24 could induce an immune response in other strains of mice, both C3H (H^{2k}) and C57BL/6 (H^{2b}) strains of mice were immunized with 50 μg peptide emulsified in complete Freund's adjuvant, then boosted with the same amount of immunogen in incomplete Freund's adjuvant. Antisera from both strains of mice were tested against HIV1 viral proteins, and showed to be able to recognize p24 and its precursor p55 in the immunoblot analysis (Fig.1A, lanes 3 and 4). These data strongly suggest that HIV1-p24 can be efficiently prevented by several MHC class II haplotypes.

To identify which residues in HIV1-p24 were critical for inducing an antibody response, a truncated peptide P24E was prepared and tested. Antisera from Balb/c mice and rabbits immunized with P24E failed to recognize p24 in both ELISA and immunoblot analyses. Although P24E failed to induce antibodies against gag-p24, it retained the ability to stimulate the proliferation of both the-p24-specific murine T-cell line (Table II) and T-lymphocytes from AIDS patients [16]. These results suggest that (i) the functional T-helper cell epitope of peptide HIV1-p24 is located within the 16 N-terminal residues; and (ii) the C-terminal sequences

Table II

Proliferative response of the HIV1-p24 specific murine T-cell line HIV-1 p24/10 to synthetic p24 peptides[a]

Peptide concentration (μg/ml)	(^{3}H)-TdR uptake (cpm)				
	HIV1-p24	P24E	BE3[c]	TAT[c]	104-mer[d]
10	2034[b]	1663	189	325	2550
2	1264	3265	176	253	1317
0.4	522	1031	132	182	756
0.08	401	N.D.[e]	130	146	831

[a] Murine lymphoblasts were harvested from spleen cells stimulated with the p24 104-mer peptide [8] and subsequently cultured in IL-2 containing medium for 7 days before stimulation with the synthetic peptides presented by irradiated (1700 R) autologous spleen cells used as antigen-presenting cells.

[b] Results are expressed as mean counts per min (cpm) of triplicate cultures. All SDs were less than 15%.

[c] BE3 and TAT served as negative control peptides. BE3 and TAT are synthetic peptides from gp160 (residues 726–750) and Tat (1–87) gene products of HIV-1, respectively.

[d] p24 104-mer corresponds to the C-terminal half of the gag p24 gene product and was prepared as previously described [8].

[e] N.D., not determined.

(TLRAEQASQEV) are essential for inducing an antibody response.

4. CONCLUSION

We have identified and synthesized a tandem T-B-cell epitope of HIV1-p24. This synthetic peptide was found to be a very potent immunogen which induced both a T-cell proliferative response and anti-p24 an antibody response in both rabbits and mice. The anti-peptide antibodies reacted with recombinant p24 in ELISA and with the mature core protein and its p55 precursor on immunoblotting. This tandem peptide should serve as a model for the rational design of a fully synthetic HIV-1 vaccine.

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