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Immunology

A complementarity-determining region synthetic peptide acts as a miniantibody and neutralizes human immunodeficiency virus type 1 *in vitro*

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ABSTRACT **A complementarity-determining region (CDR) of the mouse monoclonal antibody (mAb) F58 was constructed with specificity to a neutralization-inducing region of human immunodeficiency virus type 1 (HIV-1). The mAb has its major reactivity to the amino acid sequence I—GPGRA in the V3 viral envelope region. All CDRs including several framework amino acids were synthesized from the sequence deduced by cloning and sequencing mAb F58 heavy- and light-chain variable domains. Peptides derived from the third heavy-chain domain (CDR-H3) alone or in combination with the other CDR sequences competed with F58 mAb for the V3 region. The CDR-H3 peptide was chemically modified by cyclization and then inhibited HIV-1 replication as well as syncytium formation by infected cells. Both the homologous IIIB viral strain to which the F58 mAb was induced and the heterologous SF2 strain were inhibited. This synthetic peptide had unexpectedly potent antiviral activity and may be a potential tool for treatment of HIV-infected persons.**

Neutralizing antibodies play a crucial role in the human immune defence against the human immunodeficiency virus (HIV). There are different opinions as to the capacity of antibodies alone to prevent infection by HIV. Undoubtedly, they are important therapeutic tools during the asymptomatic period following infection. Passive immunization with neutralizing antibodies may protect from virus challenge or decrease the replication of HIV (1, 2). Eventually, the disease progresses to acquired immunodeficiency syndrome (AIDS) as new virus strains appear that escape neutralization. However, neutralizing antibodies that bind conserved amino acid sequences common to many divergent virus strains might participate to delay development of AIDS.

B-cell maturation is characterized by somatic hypermutation in antibody variable region (V) genes and selection of B cells expressing high-affinity variants of this antigen binding site (3). The mouse monoclonal antibody (mAb) F58 binds to the conserved I—GPGR sequence of V3, which is the major neutralization-inducing region of the HIV-1 outer envelope glycoprotein gp120 (4, 5). Neutralizing antibodies against the V3 top region most probably act by blocking a postbinding interaction between V3 and the host cellular membrane necessary for viral entry (6, 7). The GPGR sequence is common to most western HIV-1 virus strains (8) and F58 has been shown to have both a strong type-specific neutralization capacity as well as cross-reactive properties (4).

Complementarity-determining regions (CDRs) are hypervariable loop structures in the antigen-binding parts of antibodies and determine the specificity of antigen binding (9). Areas of amino acid hypervariability within the antigen-binding region of an antibody correspond to six loop regions, of which five have an internal canonical structure. The sixth region, the heavy (H)-chain CDR3, is more variable in both amino acid sequence and length (10). These regions combine to form an antigen-contacting groove, stabilized by a β-barrel framework. Improvement of antibody affinity may be obtained by site-directed mutagenesis of individual amino acid residues in the antibody CDRs (11). The functional binding to antigen by an antibody is strongly conformation dependent on proximity and collaboration between amino acids of these regions. Nevertheless, binding or competition by peptide sequences from selected CDRs of mAbs has recently been demonstrated (12, 13). The aim of this study was to identify the CDR region(s) of mAb F58 responsible for interacting with the HIV-1 V3 region. We also wanted to study whether specific CDR(s) could neutralize HIV-1 *in vitro*.

MATERIALS AND METHODS

Cloning of mAb F58 V_H and V_κ Genes. The V domains of the κ light and H chains of the HIV-1-neutralizing mouse mAb F58 were cloned and sequenced. Briefly, first-strand cDNA was synthesized from $\approx 10^7$ hybridoma cells by a boiling method (14). The V_H and V_κ genes were amplified from aliquots of first-strand cDNA by PCR and cloned into the vectors M13VHPCR1 and M13VκPCR1 (15). At least two clones from two separate amplifications of the V_H and V_κ genes were sequenced. The deduced amino acid sequences of the V domains were used to synthesize CDR-based peptides.

Peptides and Proteins. V3 top regions derived from four HIV strains (IIIB, MN, RF, and SF2), all CDR regions (see Table 1), and all combinations of CDRs were prepared as synthetic peptides. The combinations were prepared as continuous sequences 29–33 amino acids long. The cysteines introduced in peptides V3-MN and CDR-H3/C1–C4 were chemically oxidized to create loop structures. Synthesis was performed according to Merrifield with modifications (16–18). The recombinantly produced envelope precursor protein of HIV-1 (rgp160) was kindly donated by Frank Volvovitz (MicroGeneSys, Meriden, CT). The rgp160 was derived from the HIV-1_{IIIB} strain and was produced in cells of lepidopteran insects by using a baculovirus expression system.

Epitope Blocking by CDRs. Ninety-six-well enzyme immunoassay (EIA) plates (Nunc, Denmark) were coated with V3 peptides (1 μg per well) or rgp160 (0.1 μg per well) in 100 μl

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Abbreviations: CDR, complementarity-determining region; HIV, human immunodeficiency virus; mAb, monoclonal antibody; EIA, enzyme immunoassay; V, variable region; H chain, heavy chain; rgp160, recombinantly produced envelope precursor protein of HIV-1.

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of 0.05 M sodium carbonate buffer (pH 9.5) at room temperature overnight and stored at least 24 h at +4°C until use. CDR peptides were diluted in phosphate-buffered saline with 0.5% bovine serum albumin/0.05% Tween 20/0.01% merthiolate/2% goat serum. Each CDR peptide at 0.4–10 μg per 100 μl was added to the wells and incubated for 1 h at 37°C. An unrelated peptide from the N-terminal end of the HIV-1 envelope protein gp41 was included as a negative control. Then mAb F58 was added at 20–50 ng/ml and incubated for 1 h at 37°C. After washings, peroxidase-conjugated rabbit anti-mouse IgG (Dakopatts, Denmark) was added and incubated for 1 h at 37°C. Finally, the substrate orthophenylenediamine was added for 30 min at 37°C and the reaction was stopped by adding 2.5 M H_2SO_4. The absorbance was measured at 490 nm. A 50% decrease of mAb F58 binding was considered significant. In a modification of the assays described above, 10 ng to 10 μg of CDR peptide and 2 ng to 50 μg of mAb F58 were simultaneously added to EIA wells coated with V3 peptide or rgp160. Binding of mAb F58 was indicated as described above. As a control experiment, the CDR peptides were also assayed for inhibition of binding between the anti-HBe/β mouse mAb 57/8 and its hepatitis B epitope HBe 128–133 (19). In each blocking and competition assay, the mAb F58 was first titrated for binding to coated V3 peptide or gp160 to give absorbance values between 0.2 and 1.0.

mAb F58 Binding by Anti-Idiotypes. To generate anti-idiotypic mAbs against mAb F58, BALB/c mice (Harlan–Sprague–Dawley) were immunized subcutaneously with 20 μg of mAb F58 $F(ab')_2$ per injection coupled to keyhole limpet hemocyanin. The number of injections and the adjuvant used varied for the various anti-idiotypes produced [anti-idiotypes MF3H12, MF2B10, and MG2H3: first immunization in complete Freund's adjuvant, second to sixth immunizations in incomplete Freund's adjuvant; anti-idiotypes ML5H10, MN4A7, MN5B11, and MM5E1: one immunization in Titer Max adjuvant (CytRX, Norcross, GA)]. All mice seropositive for anti-idiotypic antibodies were injected intravenously with 20 μg of F58 $F(ab')_2$ (anti-idiotype MG2H3) or F58 $F(ab')_2$ keyhole limpet hemocyanin (all other anti-idiotypes) 3 days before fusion of splenocytes to SP2/0 mouse myeloma cells following standard protocols using polyethylene glycol 1500 and hypoxanthine/aminopterin/thymidine-containing selection medium. Hybridoma culture supernatants diluted 10^{-1}–10^{-5} were incubated with mAb F58 for 1 h at 37°C. This mixture was added to the wells of a V3-IIIB-coated EIA plate and incubated for 1 h at 37°C. The following procedures were the same as described above. Tissue culture supernatants were also tested for binding to the six CDRs of mAb F58 using EIA plates coated with 1 μg of CDR peptide per well. Supernatants were used in dilutions of 10^{-2} and 10^{-3}.

***In Vitro* HIV-1 Neutralization.** CDR peptides (50 ng to 50 μg) were incubated with HIV-1_{IIIB} or HIV-1_{SF2} in 100 μl per EIA well for 1 h at 37°C. Each peptide was tested in duplicate, 50 ng to 50 μg per well of F58 was included as a positive control, and 10^4 cells of human $CD4^+$ T-cell line C8166 were added to each well and incubated for 1 h at 37°C in 200 μl per well. The cells were washed once in RPMI 1640 medium containing 10% heat-inactivated fetal calf serum and then incubated in 200 μl of medium per well for 6 days at 37°C and 5% CO_2/95% air. After 6 days, the neutralizing capacity of the CDR peptides was determined by measuring HIV-1 p24 antigen content in cell culture supernatants by capture EIA, by microscopic evaluation of syncytia, and by immunofluorescence. A 50% reduction of p24 content or of the number of syncytia was defined as neutralization (IC_{50}). Fifty micrograms of each CDR peptide was incubated with cells for 1 h at 37°C to detect possible toxic effects. Trypan blue exclusion and the number of cells were evaluated after 6 days.

RESULTS

Epitope Blocking. Peptides based on CDR1 of the V_κ domain and CDR2 of the V_H domain of mAb F58 included only the CDR sequence. The other CDR peptides included neighboring framework residues in addition to the CDR residues (Table 1). These additions were made to obtain peptides of similar lengths. The CDR peptides were used to directly block the V3 epitope of HIV-1 represented by peptide V3-IIIB. Only the peptide CDR-H3, representing the third H-chain CDR of mAb F58, and its combinations with sequences representing the other five CDR regions could block the binding of mAb to V3-IIIB (Fig. 1*A*). The best inhibition was obtained with the combined peptide representing CDR-H2/H3.

Competition Between mAb F58 and CDRs. CDR peptides and mAb F58 were allowed to compete for binding to viral antigens represented by recombinant gp160 or V3 peptides. CDR-H3 and all its combinations competed with the mAb for binding to the antigens (data not shown). CDR-H3 and its cyclic analogues (Table 1) competed well with mAb F58 for gp160 epitopes (Fig. 1*B*) and heterologous V3 sequences represented by peptides V3-MN, V3-RF, and V3-SF2 (Fig. 1*C*). Interestingly, CDR-H1 and CDR-H2 by themselves instead enhanced binding of mAb F58 to gp160 (Fig. 1*B*), indicating their independent interaction with the V3 region. All cyclic CDR-H3 peptides competed with mAb F58 better than the linear CDR-H3 (Fig. 1 *B* and *C*). None of our anti-HIV mAb F58-based CDR peptides could inhibit binding between the anti-HBe/β mAb 57/8 and its epitope, verifying the specificity of our competition results.

Inhibition of mAb F58 by Anti-Idiotypes. Mouse anti-idiotypic antibodies bound exclusively to mAb F58 but not to normal mouse IgG (results not shown). The anti-idiotypes were used to inhibit the mAb F58 binding to peptide V3-IIIB. Six of seven different anti-idiotypic hybridoma culture supernatants showed >50% inhibition at a dilution of 10^{-2}. To determine whether the inhibition was caused by direct binding of the anti-idiotypic antibodies to antigen-interacting regions of mAb F58, we assayed the supernatants for binding to the six CDR peptides. No binding of any of the seven anti-idiotypes to any of the CDRs could be detected.

Inhibition of HIV-1 Replication. CDR peptides (0.5 μg to 0.5 mg/ml) were used to neutralize or inhibit syncytium formation by HIV-1_{IIIB} or HIV-1_{SF2}. The cyclic forms of the CDR-H3 peptide neutralized HIV better than the linear form (Table 2 and Fig. 2). Syncytium inhibition was obtained with the two viral strains IIIB and SF2 (Fig. 3). Again the cyclic peptides

Table 1. Synthetic V3 and CDR peptides

Peptide(s)	Amino acid sequence
V3-IIIB	RKSIRIQRGPGRAFV
V3-MN	KRKCIHIGPGRAFYCTK * *
V3-RF	RKSITKGPGRVIYATG
V3-SF2	RKSIYIGPGRAFHTTG
CDR-L1	RASESVDDYGISFMH
CDR-L2	LLIYRASNLESGIPA
CDR-L3	YYCQQSNKDPLTFG
CDR-H1	GYTFTDHIMNWVKKR
CDR-H2	RIFPVSGETNYNQKFMG
CDR-H3	CDLIYYDYEEDYYFDY
CDR-H3/C1	CDLIYYDYEEDYYFDYC * *
CDR-H3/C2	CDLIYYDYEEDYYFDC * *
CDR-H3/C3	CDLIYYDYEEDYYFC * *
CDR-H3/C4	CDLIYYDYEEDYYC * *

Underlined sequences are CDRs of the mAb F58. Parts of framework regions (not underlined) were included. *, Disulfide bridges between native or introduced cysteines formed by chemical oxidation to create cyclic peptides.

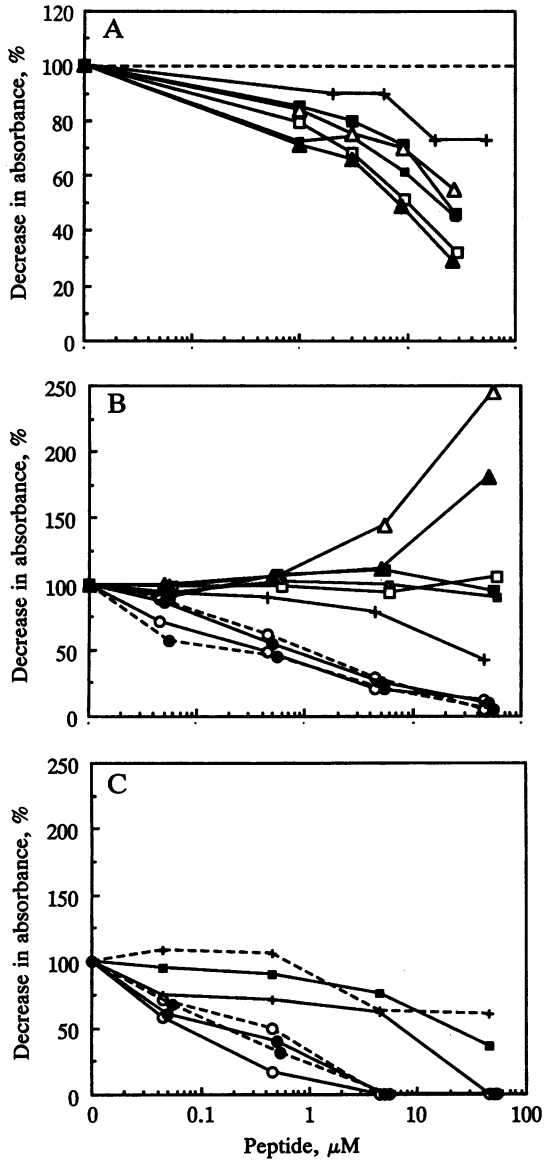

FIG. 1. (*A*) Blocking of the mouse mAb F58 from binding to the HIV-1 epitope peptide V3-IIIB by the CDR-H3 peptide and its peptide combinations. +, CDR-H3; ⊡, CDR-L1/H3; □, CDR-L2/H3; ■, CDR-L3/H3; △, CDR-H1/H3; ▲, CDR-H2/H3. Dotted line indicates mAb F58 binding. (*B*) Competition between mAb F58 and CDR peptides for binding to recombinant HIV-1 envelope protein rgp160. ⊡, CDR-L1; □, CDR-L2; ■, CDR-L3; △, CDR-H1; ▲, CDR-H2; +, CDR-H3; ○—○, CDR-H3/C1; ○----○, CDR-H3/C2; ●—●, CDR-H3/C3; ●----●, CDR-H3/C4. (*C*) Competition between mAb F58 and CDR peptides for binding to V3 peptides V3-MN, V3-RF, or V3-SF2. ■, CDR-H3 (V3-RF); +----+, CDR-H3 (V3-SF2). The following CDR peptides were tested against V3-MN: +—+, CDR-H3; ○—○, CDR-H3/C1; ○----○, CDR-H3/C2; ●—●, CDR-H3/C3; ●----●, CDR-H3/C4.

were more active than the linear CDR-H3 in reducing syncytium formation with both viral strains. The strongest syncytium-inhibiting effects were seen against the homologous IIIB viral strain but a clear inhibition of the heterologous SF2 strain was also seen (Fig. 3). The CDR-H1 peptide appeared to be toxic since no cells survived 1 h of peptide incubation together with 6 days of cultivation. The other CDR peptides did not affect the viability or proliferation of the cells.

DISCUSSION

We have identified a CDR sequence that specifically inhibits the HIV-1-neutralizing antibody from which it originates

Table 2. Neutralizing capacity of CDR peptides determined by p24 EIA

Peptide(s)	Neutralization* IIIB virus	SF2 virus
CDR-H3	0.60 (274)	>0.50 (>228)
CDR-H3/C1	0.38 (166)	0.43 (188)
CDR-H3/C2	0.30 (141)	0.38 (178)
CDR-H3/C3	0.35 (174)	0.78 (387)
CDR-H3/C4	0.40 (214)	0.70 (375)

*IC_{50}, mg/ml (μM).

from binding to its antigen. The results clearly indicate that the sequence CDLIYYDYEEDYYFDY in CDR-H3 is a major part of the discontinuous determinant involved in the antibody–antigen interaction. Mass spectroscopic analysis of the linear CDR-H3 peptide revealed that a fraction of the crude product consisted of the correct amino acid sequence. An interesting feature of this third hypervariable loop of the H chain is that it has a large number of tyrosines and negatively charged residues, whereas the V3 antigen predominantly has positively charged residues.

In native form as part of an antibody structure, CDRs form loops usually stabilized by β turns (20). After cyclic modifications, we improved the binding of CDR-H3 to V3 epitopes and virions compared to the linear CDR-H3. This is perhaps due to stabilization of a β turn not predominant in the linear peptide (21).

CDR-H3 interacted with V3 peptides of several HIV subtypes, thus inhibiting the reactivity of mAb F58 in blocking experiments. All combinations with CDR-H3 blocked mAb F58, the most competent being CDR-H2/H3. In competition experiments, single CDR-H3 had an advantage, perhaps because of its higher affinity or its smaller molecular size. An interesting finding was that CDR-H1, and to some extent CDR-H2 when alone, caused enhanced binding of mAb F58 to gp160. This may be taken to indicate an interaction of CDR-H1 with V3 outside of the GPGR sequence to enhance the GPGR availability to the mAb. Such a phenomenon indicates that single small peptides may be able to change the conformation of the protein to allow better binding to a definite site, in this case mAb F58 binding to GPGR. Another explanation may be that the CDR-H1 peptide has a general protein binding ability since it was toxic to our cell cultures.

The 14- to 17-amino acid residues in linear and cyclic forms representing CDR-H3 also neutralized HIV-1 *in vitro* and inhibited virus-induced syncytia. The concentration of linear CDR-H3 critical for neutralization appeared to be 0.6 mg/ml or 274 μM. It seemed that the peptide was more effective in

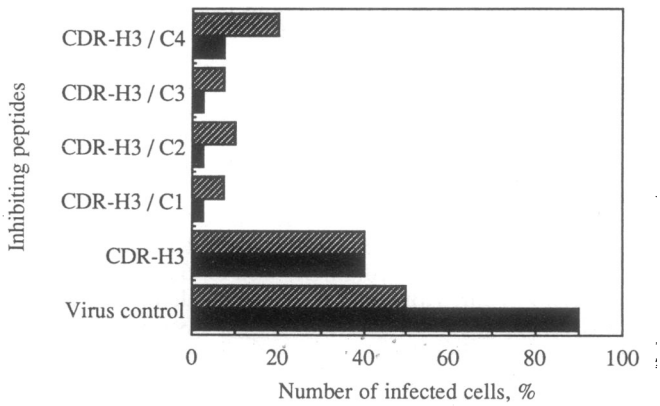

FIG. 2. Neutralizing capacity of 0.5 mg (220–270 μM) of CDR peptide per ml in C8166 cell cultures infected by $HIV\text{-}1_{IIIB}$ or $HIV\text{-}1_{SF2}$ determined by immunofluorescence. □, SF2 virus; ■, IIIB virus.

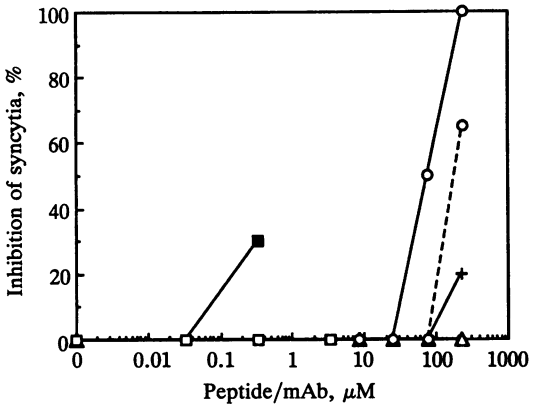

FIG. 3. Capacity of CDR-H3 and CDR-H3/C2 peptides and mAb F58 to inhibit syncytia of human C8166 T cells induced by HIV-1_{IIIB} and HIV-1_{SF2} determined by microscopy. All four cyclic CDR-H3 peptides had the same inhibiting capacity (data not shown). +, CDR-H3 inhibiting HIV-1 (IIIB); △, CDR-H3 (SF2); ○—○, CDR-H3/C2 (IIIB); ○----○, CDR-H3/C2 (SF2); ■, F58 (IIIB); □, F58 (SF2).

preventing syncytium induction by cell-to-cell spread of virus than by neutralizing free virions. During the fusion process that initiates syncytium formation between infected cells, the CDR binding to viral sequences may be more effective than on virions. Previously, mAb F58 has been shown to mediate type-specific neutralization in a concentration of 0.3–70 nM, depending on cell type and culture conditions. Cross-reactive neutralization required up to 3.3 μM mAb F58, depending on viral strain (4). Compared to our CDR peptides, the type-specific neutralization by whole F58 mAb thus occurs in a 10^2- to 10^3-fold lower concentration. It was obvious that modification of the CDR-H3 peptide by cyclization increased its binding capacity to antigen. We assume that this was due to a stabilization of the minimal recognition unit within this CDR that could not be further improved by shortening the cyclic peptide.

A biologically active peptide that inhibits reovirus 3 from attaching to its receptor was identified by Williams *et al.* (20). This peptide was derived from the second light-chain CDR of an anti-receptor mAb, while our active peptide is from CDR-H3 of mAb F58. Our results are not unexpected, though, since CDR3 appears to be the most variable of the CDRs in mouse V_H (9).

CD4 binding of virion gp120 is the first step in HIV-1 infection. The neutralization by antibodies has been suggested to be due to blocking of subsequent conformational changes, perhaps by blocking a protease cleavage site. Hattori *et al.* (6) suggested that cellular proteinases cleave the viral envelope glycoprotein, enabling the virus to enter the cell. A recombinant construction of the cellular proteinase tryptase TL_2 binds specifically to the envelope protein gp120. To elucidate whether our anti-V3 mAb F58 had any sequences in common with the cellular tryptase, a computer search for amino acid homology was performed with the CDR-H1 and -H3 regions of mAb F58. No homology was identified except in framework sequences.

Short peptides representing the V3 region itself have also been shown to inhibit HIV-1 infection *in vitro* (Vahlne, A., Horal, P., Hall, W., Rymo, L., Czerkinsky, C., Holmgren, J. & Svennerholm, B., Abstract, Annual Meeting of HIV and Retroviruses, National Cancer Institute, Washington, August 8–15, 1992).

The amino acids of the V3 region essential for V3-F58 binding have been described as I—GPGRA (22), common to many western HIV-1 strains (8). It may therefore be possible to delay progression to AIDS in HIV-1-infected individuals or even to prevent primary infection by CDR administration.

There should be several advantages of using CDR peptides instead of complete antibodies for passive immunization of HIV patients. The use of peptides would minimize secondary anti-idiotypic immune responses, higher molar concentrations could be given, and peptides would also penetrate better and to more compartments of the human body. One problem in passive immunization is the high possibility of secondary immune responses against the idiotopes of immunizing antibodies leading to rapid elimination of the antibody. As mentioned, such secondary responses may be reduced by immunizing only with the CDRs of the antibody. Anti-idiotypic antibodies (mouse anti-mAb F58) were studied to evaluate whether secondary immune responses could influence antibody–antigen interaction and consequently reduce mAb neutralizing capacity. Such inhibition of mAb F58 was seen in our mouse model, but the anti-idiotypes did not bind to any of the six linear CDRs. Their specificity may be directed to conformational CDR idiotopes. It is also possible that they bind to idiotopes located in the framework outside the hypervariable regions. The inhibition of mAb F58 interaction with antigen is then most probably due to steric hindrance of the antigen-interacting regions and not through direct binding to these regions.

Two major conclusions can be made: It is possible to inhibit the infectivity of HIV-1 by using a synthetic peptide corresponding to a H-chain CDR of a neutralizing antibody. This peptide appears to be cross-reactive since it inhibited the heterologous SF2 strain, differing in the V3 sequence from the IIIB strain to which mAb F58 was induced. The GPGR sequence is common between these strains and a broad neutralization of primary strains has previously been demonstrated (4). The results of this study reveal an additional field of application of synthetic peptides in the treatment of HIV-1 infection as biologically active miniantibodies.

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1. Wahren, B., Bratt, G. & Hinkula, J. (1991) 5:e Colloque des Cent Gardes, October 29–31 (Pasteur Mérieux, Paris), pp. 263–267.
2. Emini, E. A., Schleif, W. A., Nunberg, J. H., Conley, A. J., Eda, Y., Tokiyoshi, S., Putney, S. D., Matsushita, S., Cobb, K. E., Jett, C. M., Eichberg, J. W. & Murthy, K. K. (1992) *Nature (London)* **355**, 728–730.
3. Kocks, C. & Rajewsky, K. A. (1989) *Annu. Rev. Immunol.* **7**, 537–559.
4. Åkerblom, L., Hinkula, J., Broliden, P. A., Mäkitalo, B., Fridberger, T., Rosen, J., Villacres-Ericsson, M., Morein, B. & Wahren, B. (1990) *AIDS* **4**, 953–960.
5. Rusche, J. R., Javaherian, K., McDanal, C., Petro, J., Lynn, D. L., Grimaila, R., Langlois, A., Gallo, R. C., Arthur, L. O., Fischinger, P. J., Bolognesi, D. P., Putney, S. D. & Matthews, T. J. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 3198–3202.
6. Hattori, T., Koito, A., Takatsuki, K., Kido, H. & Katunuma, N. (1989) *FEBS Lett.* **248**, 48–52.
7. Freed, E. O., Myers, D. J. & Risser, R. (1991) *J. Virol.* **65**, 190–194.
8. Myers, G. (1991) *Database of Human Retroviruses and AIDS* (Los Alamos Natl. Lab., Los Alamos, NM).
9. Kabat, E. A. (1987) *Sequence of Proteins of Biological Interest* (U.S. Dept. of Health and Human Services, Natl. Inst. of Health, Bethesda, MD).
10. Chothia, C., Lesk, A. M., Tramontano, A., Levitt, M., Smith-Gill, S. J., Air, G., Sheriff, S., Padlan, E. A., Davies, D., Tulip, W. R., Colman, P. M., Spinelli, S., Alzari, P. M. & Poljak, R. J. (1989) *Nature (London)* **342**, 877–883.
11. Roberts, V. A., Iverson, B. L., Iverson, S. A., Benkovic,

S. J., Lerner, R. A., Getzoff, E. D. & Tainer, J. A. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 6654–6658.
12. Welling, G. W., Van Gorkum, J., Damhof, R. A. & Drijfhout, J. W. (1991) *J. Chromatogr.* **548**, 235–242.
13. Taub, R., Gould, R. J., Garsky, V. M., Ciccarone, T. M., Hoxie, J., Friedman, P. A. & Shattil, S. J. (1989) *J. Biol. Chem.* **264**, 259–265.
14. Ferre, F. & Garduno, F. (1989) *Nucleic Acids Res.* **17**, 2141.
15. Orlandi, R., Gussow, D. H., Jones, P. T. & Winter, G. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 3833–3837.
16. Merrifield, R. B. (1963) *J. Am. Chem. Soc.* **85**, 2149.
17. Houghten, R. A. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 5131–5135.
18. Sällberg, M., Rudén, U., Magnius, L. O., Norrby, E. & Wahren, B. (1991) *Immunol. Lett.* **30**, 59–68.
19. Sällberg, M., Rudén, U., Wahren, B., Noah, M. & Magnius, L. O. (1991) *Mol. Immunol.* **28**, 719–726.
20. Williams, W. V., Moss, D. A., Kieber-Emmons, T., Cohen, J. A., Myers, J. N., Weiner, D. B. & Greene, M. I. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5537–5541.
21. Williams, W. V., Kieber-Emmons, T., VonFeldt, J., Greene, M. I. & Weiner, D. B. (1991) *J. Biol. Chem.* **266**, 5182–5190.
22. Broliden, P. A., Mäkitalo, B., Åkerblom, L., Rosen, J., Broliden, K., Utter, G., Jondal, M., Norrby, E. & Wahren, B. (1991) *Immunology* **73**, 371–376.