Characterization of a V3 domain-specific neutralizing human monoclonal antibody that preferentially recognizes non-syncytium-inducing human immunodeficiency virus type 1 strains

M. Schutten,[1] J. P. M. Langedijk,[2] A. C. Andeweg,[3]† R. C. Huisman,[1] R. H. Meloen[2] and A. D. M. E. Osterhaus[1]*

[1] *Institute of Virology, Erasmus University Rotterdam, Dr Molewaterplein 50, PO Box 1738, 3000 DR Rotterdam,* [2] *Laboratory for Molecular Immunology, Central Veterinary Institute, Edelhertweg 15, 8200 AB Lelystad and* [3] *Laboratory of Immunobiology, National Institute of Public Health and Environmental Protection, A. van Leeuwenhoeklaan 9, 3720 BA Bilthoven, The Netherlands*

A type-specific human immunodeficiency virus type 1 (HIV-1)-neutralizing human monoclonal antibody (HuMAb MN215) is described that reacts with the V3 domain of a number of subtype B virus strains. Pepscan analysis indicated that amino acids at both sides of the tip of the V3 loop were involved in the binding of HuMAb MN215. The minimum epitope in a V3 sequence, obtained from the donor from whom the cell line originated, was 9 amino acids long and proved to be located at the C-terminal side of the tip of the loop. In a replacement Pepscan analysis, individual amino acids of the V3 loop important for binding of HuMAb MN215 were identified. Amino acids at positions 15 (H), 16 (I), 17 (G) and 18 (P) were found to be essential for binding of the antibody, whereas changes at positions 19 of G to N, 20 of R to K and 23 of F to L, as well as the addition of a negative charge at the C terminus, improved binding. Thus, amino acids involved in the binding of HuMAb MN215 are primarily located within highly variable regions of the V3 loop. HuMAb MN215 showed a higher affinity for the V3 domain sequences and recombinant envelope glycoproteins derived from non-syncytium-inducing strains than for those derived from syncytium-inducing strains.

Introduction

Virus neutralizing (VN) antibodies are believed to play a major role in the pathogenesis of human immunodeficiency virus type 1 (HIV-1) infection. Suppression of virus replication during the relatively long asymptomatic period is believed to be mediated at least in part by the humoral immune response (Page *et al.*, 1992; Watkins *et al.*, 1993). The identification and characterization of antigenic sites on the glycoproteins of HIV-1 that elicit VN antibodies therefore seems to be a prerequisite for the rational development of passive and active immunization strategies. The third variable domain (V3 domain) of the outer membrane glycoprotein (gp120) of HIV-1 has been shown to elicit VN antibodies (Goudsmit *et al.*, 1988; Palker *et al.*, 1988). Despite the variability within this antigenic site, certain antibodies directed against this domain may neutralize a wide range of virus strains (Langedijk *et al.*, 1991; Ohno *et al.*, 1991; Gorny *et al.*, 1992). Certain human monoclonal antibodies (HuMAbs) directed against the V3 domain are therefore likely candidates for passive immunization therapies. A mouse monoclonal antibody directed against the V3 domain of the HIV-1 IIIB strain has been shown to protect a chimpanzee against a challenge with the homologous virus strain when the antibody was administered prior to challenge (Emini *et al.*, 1992).

The V3 domain has been shown to be an important denominator for the phenotype of the virus: it directly influences the syncytium-inducing (SI) capacity and cell tropism of the virus. Mutations in the V3 domain of HIV-1 strains or the exchange of this region between highly related but phenotypically distinct glycoproteins may change their biological phenotypes (Freed & Risser, 1991; de Jong *et al.*, 1992; Andeweg *et al.*, 1993). It has also been shown that the V3 domain consensus sequence of non-syncytium-inducing (NSI) HIV-1 strains differs in several aspects from the V3 consensus sequence of SI HIV-1 strains (Fouchier *et al.*, 1992). A similar phenomenon has been observed when the V3 domain consensus sequence of macrophage-tropic and T cell-tropic HIV-1

* Author for correspondence. Fax +31 10 4365145. e-mail osterhaus@viro.fgg.eur.nl

† Present address: BPRC, Lange Kleiweg 151, 2288 GJ Rijswijk, The Netherlands.

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strains were compared (Chesebro *et al.*, 1992). Therefore, it may be expected that certain antibodies directed against this site display different reactivities with HIV-1 strains of different phenotypes. HIV-1 strains isolated during the asymptomatic period of HIV-1 infection are predominantly of the NSI phenotype and are therefore considered important targets for early passive immunization therapies (Roos *et al.*, 1992; Zhang *et al.*, 1992; Zhu *et al.*, 1993). Studies with V3 domain-specific HuMAbs have primarily focused on their reactivities with HIV-1 strains of the SI phenotype, since these HIV-1 strains are most commonly used in assay systems. Here we describe the generation and characterization of a HIV-1 V3 domain-specific HuMAb that reacts predominantly with HIV-1 strains of the NSI phenotype.

Methods

Generation of a human B cell clone producing V3 domain-specific HuMAb. Peripheral blood mononuclear cells (PBMC) were isolated from 40 ml heparinized blood of an HIV-1-seropositive asymptomatic adult male of the Amsterdam cohort (donor #658) four years after seroconversion; generation of Epstein–Barr virus (EBV)-transformed B cell lines was performed essentially as previously described (Teeuwsen *et al.*, 1990; Schutten *et al.*, 1993). The B cell line described in the present paper was fused with the mouse–human heterohybridoma F3B6 (Foung *et al.*, 1984) and subcloned several times, after which it was expanded into flasks.

For the identification of antibodies in culture supernatant, ELISA plates (Costar) were coated with a 23 amino acid peptide, purchased from American BioTechnologies, spanning the tip of the V3 loop of HIV-1 MN gp120. This peptide was coated overnight at 4 °C in PBS pH 7·5 at 750 ng/ml. The ELISA was developed as previously described (Schutten *et al.*, 1993). Twelve peptides representing the V3 region of HIV-1 subtype B [Universal (subtype B consensus; Meyers *et al.*, 1992), MN, SC, SF2, CDC4, HXB2, WM52, RF and NY/5 strains] and subtype D (ELI, Z2 and Z6 strains) (Meyers *et al.*, 1992) were purchased from American BioTechnologies and coated under the same conditions for determination of the cross-reactivity. IgG subclass determination was carried out in ELISA with subclass-specific mouse monoclonal antibodies (Seralab). The HIV-1 gp120 V3 loop sequence from virus in the serum of donor #658 was determined as described previously (Wolfs *et al.*, 1991).

Peptide inhibition ELISA. HuMAb MN215 was incubated with serial dilutions of the HIV-1 Universal, MN, SF2 and SC V3 peptides at a concentration at which half of the maximum binding was achieved in a solid phase V3 loop peptide ELISA using the HIV-1 MN V3 peptide. Furthermore, peptides generated by Dr C. Sia (Connaught Laboratories, Canada) were used in this assay. These peptides were generated on the basis of consensus SI and NSI sequences obtained from a previously described set of eight recombinant envelop proteins derived from two donors (#16 and #320) from the Amsterdam cohort (Fig. 1; Andeweg *et al.*, 1992). This set consisted of four recombinant glycoproteins per donor, of which two were derived from viruses with the SI phenotype and two from viruses with the NSI phenotype. ELISA plates coated with the HIV-1 MN V3 peptide were incubated for 1 h at 37 °C with these HuMAb MN215–peptide mixtures, which were preincubated overnight at 4 °C. The ELISA was completed as described above.

Pepscan analysis. Pepscan analysis was performed as originally described by Geysen *et al.* (1984) and modified by Langedijk *et al.* (1991). In short, peptides were synthesized on polyethylene rods and tested with HuMAb MN215 in an ELISA according to established procedures (Langedijk *et al.*, 1991). A set of peptides of between 8 and 16 amino acids in length, with all but 1 amino acid overlapping, were synthesized with sequences derived from the HIV-1 MN gp120 V3 loop

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                                10                  20                  30
                                |                   |                   |
Universal V3*          C T R P N N N T R K S I H I  G P G R A F Y T T G E I I G D I R Q A H C
#658-3                         . . . . . G . . .    . . . . . .
#658-27                        . . . . . G . . .    . . . . . . . . A . D . . . . N
Dutch consensus                . . . . . .          . . . . . .
#16 SI*                        . . . . . G . . .    . . . . . V . . . . R . . . . . . . . .
#16 NSI*                       . . . . . G . . .    . . . . . .
#320 SI*                       . . . . . G . . .    . . . . . . . A A R K . . . . . . . . . .
#320 NSI*                      . . . . . G . . M    . . . K . . . A . . Q . . . . . . . . . .
MN*                            C . K . . R . . .    . . . . . K N
SC*                            C . . T R . . . .    . . . . . A . . D
SF2*                           C . . . . . Y .      . . . . . H . . . R
CDC4*                          C H . . . R V T L    . . . . V W . . . .
HXB2*                          C . . . . R . R . Q R . . . . . V . I . K
NY/5*                          C . . K . G . A .    . . . . T L . A R E K
Z2*                            C . I . Q R T S .    . L . Q . L . . . K T R S
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Fig. 1. Comparison of HIV-1 V3 domain amino acid sequences. The asterisk indicates V3 sequences from different HIV-1 strains from which peptides were used in solid phase peptide and peptide inhibition ELISA. The Dutch consensus, #658–3 and #658–27 V3 sequences were used in Pepscan.

(Fig. 1; Meyers *et al.*, 1992) and one sequence more related to the Dutch consensus V3 sequence (Fig. 1; Holley *et al.*, 1991). To determine the individual amino acid which was important for binding of HuMAb MN215 to its epitope, peptide analogues were made from the Dutch V3 consensus sequence (from position 12 to 27) by substituting single amino acids. The amino acids within this sequence were substituted for amino acids which have been proven to be prevalent at each position in Dutch HIV-1 isolates (Holley *et al.*, 1991) or by amino acids with different biochemical properties. Numbering of the amino acids in the HIV-1 gp120 V3 loop was according to LaRosa *et al.* (1990).

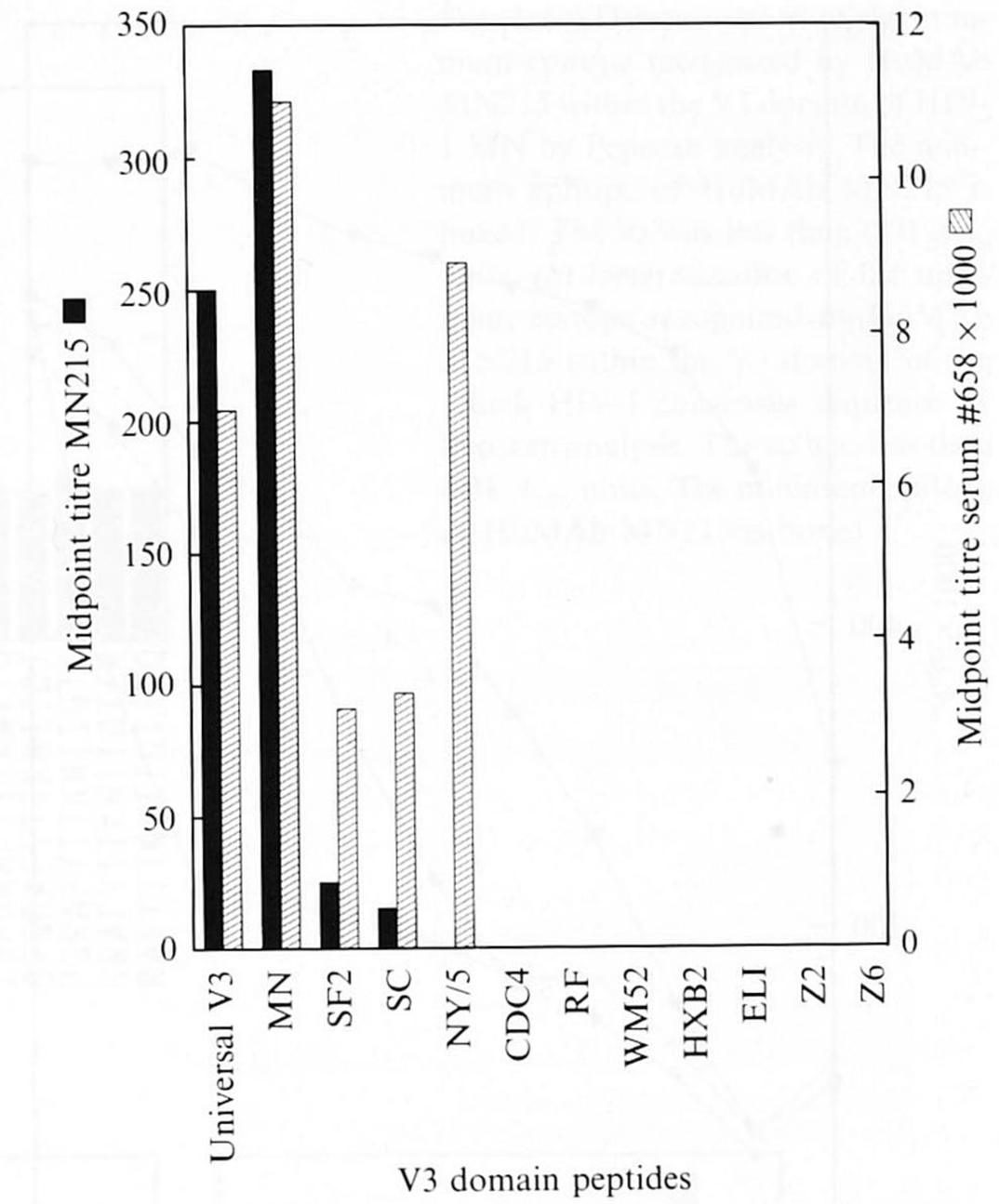
Relative affinity of monoclonal antibodies for HIV-1 gp160. Recombinant HIV-1 envelope glycoproteins, which we have described recently (Andeweg *et al.*, 1992), were expressed in HeLa cells by a recombinant vaccinia virus (rVV) expression system. The rVVs used were 320–2a.5 and 320–2a.7 (donor #320; phenotype SI), 320–2a.3 and 320–2a.6 (donor #320; phenotype NSI), 16.1 and 16.2 (donor #16; phenotype SI), and 16.3 and 16.4 (donor 16; phenotype NSI) (Andeweg *et al.*, 1992). At 24 h after rVV infection, HeLa cells were lysed with 1% empigen in PBS, in the presence of proteinase inhibitors (10 μg/ml aprotinin, 1 mM-PMSF; Boehringer Mannheim). For HIV-1 MN glycoproteins, persistently infected SupT1 cells were cocultured with uninfected SupT1 cells at a ratio of 1:4. After 3 days cells were centrifuged and lysed as described above. Cell lysates were divided into aliquots and the amount of gp120/160 was determined in a catching antibody ELISA as previously described (Moore, 1990). Equal amounts of gp120/160 were coated to ELISA plates and the concentration where 50% of the maximum A_{450} was reached with the HuMAbs was taken as a relative measure for affinity. The HuMAbs directed against more conserved sites on gp160 used in this ELISA have been previously described (Teeuwsen *et al.*, 1990; Gorny *et al.*, 1993; Schutten *et al.*, 1993).

Virus entry inhibition assay. The *env* gene encoding the recombinant 16.2 SI glycoprotein was cloned into an expression vector as previously described (Andeweg *et al.*, 1993) and subsequently used to generate chimeric infectious virus using a *trans* complementation assay. In short, an *env*-defective HIV-1 provirus, encoding the bacterial CAT reporter gene, was complemented by the 16.2 SI *env* gene for a single round of replication by co-transfection into the $CD4^-$ COS cell line. This resulted in the production of virions carrying heterologous envelope glycoproteins which could infect $CD4^+$ cells like the SupT1 cells used in this study (Helseth *et al.*, 1990). After 3 days cell-free supernatant was collected by centrifugation and frozen at −135 °C. For infection the amount of p24 antigen, as determined with a p24 ELISA kit (V5; Organon Teknika), was calibrated and the level of envelope expression was checked with the capture antibody ELISA (see above). Infection was performed overnight at 37 °C in 1 ml medium. After 24 h the cells were washed and cultured for 72 h. CAT activity in these cells was measured in a previously described CAT assay using a two-phase extraction system (Seed & Sheen, 1988). CAT activities of the cells were considered a direct measure of virus infectivity in these assays.

Results

Generation of HuMAb MN215

Screening of the supernatants of more than 2000 B cell lines obtained by EBV transformation of PBMC collected from donor #658 and subcloning of positive cell cultures yielded one B cell line (MN215) producing an IgG1 antibody that recognized a peptide with the V3 domain sequence of the HIV-1 MN strain. This B cell line continued to produce these V3 domain-specific antibodies at levels of 4 μg/ml after fusion with the mouse–human heterohybridoma F3B6 and further subcloning. The cell line was considered clonal on the basis of the limiting dilution procedure used (Schutten *et al.*, 1993).



MN215 ■	250	333	25	15	0	0	0	0	0	0	0	0
Serum 658 ▨	7	11	3·1	3·3	8·9	0	0	0	0	0	0	0

Fig. 2. Reactivities of HuMAb MN215 and serum from donor 658 with different HIV-1 V3 peptides. Midpoint titres of HuMAb MN215 (filled bars) and serum taken from donor 658 4 years after seroconversion (hatched bars), at which 50% of maximum A_{450} was reached in an ELISA with V3 domain peptides from different subtype B (Universal, MN, SF2, SC, NY/5, CDC4, RF, WM52, HXB2) and subtype D (ELI, Z2, Z6) HIV-1 strains (see Fig. 1).

Cross reactivity of HuMAb MN215 with different HIV-1 V3 domain peptides

V3 loop sequences of HIV-1 derived from donor #658 at 3 and 60 months after seroconversion (#658–3 and #658–27, respectively) proved to be closely related to the subtype B HIV-1 V3 loop consensus sequence (Fig. 1). When tested in a solid phase peptide ELISA with V3 domain peptides representing subtype B and D HIV-1 strains, the serum from donor #658 taken 4 years after seroconversion showed specificity for peptides derived from subtype B HIV-1 strains (Fig. 2). We subsequently tested HuMAb MN215 for its reactivity in a solid phase

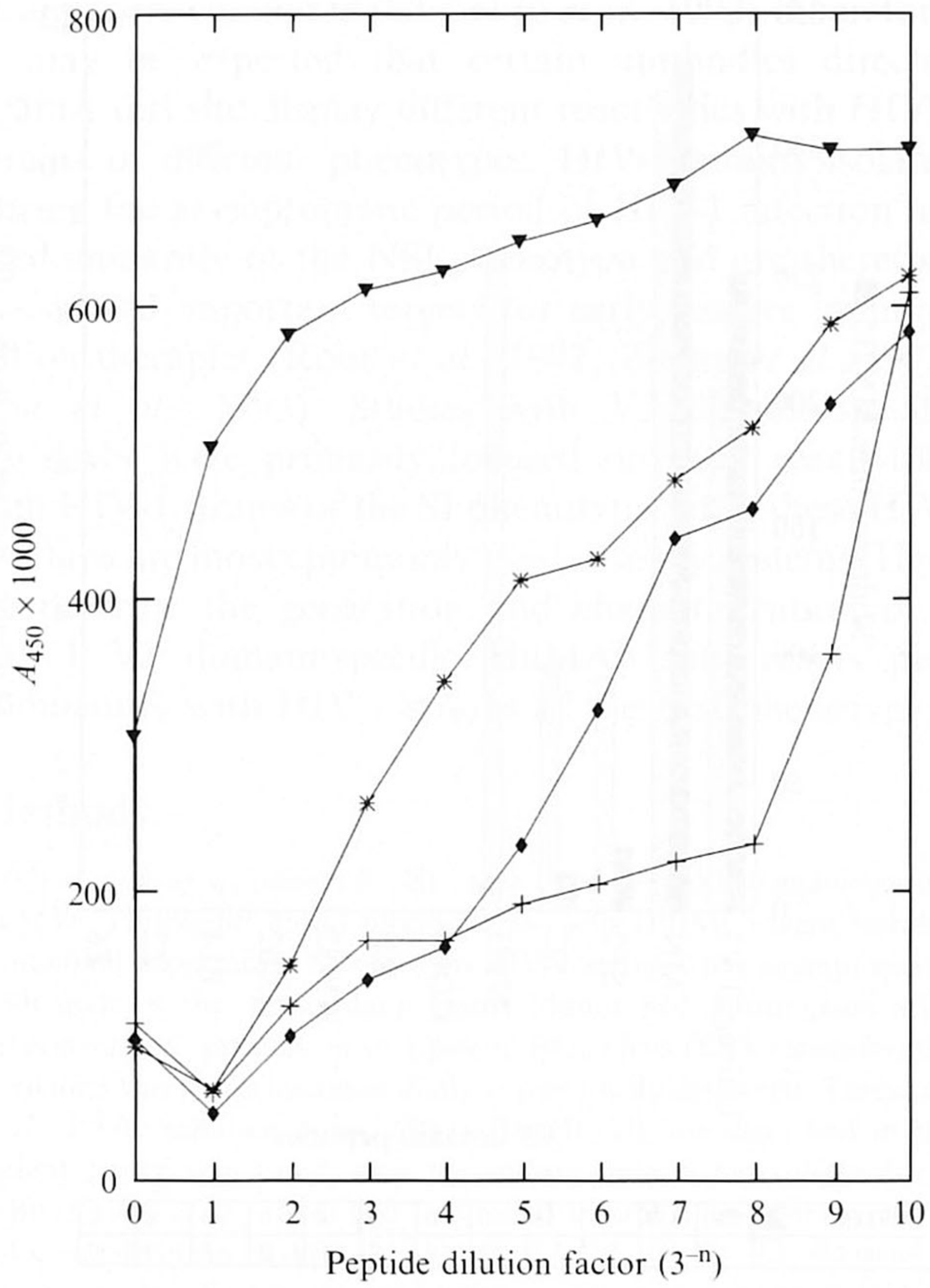


Fig. 3. Peptide inhibition ELISA of HuMAb MN215 with V3 loop peptides. A_{450} values obtained in a solid phase HIV-1 MN V3 peptide ELISA with a standard concentration HuMAb MN215, incubated with serial threefold dilutions of the respective peptides [HIV-1 Universal (◆), MN (+), SC (*), SF2 (▼); see Fig. 1] using a starting peptide concentration of 3×10^{-11} mol.

peptide ELISA with peptides derived from different subtype B HIV-1 strains and with peptides derived from subtype D HIV-1 strains (Fig. 2). The highest reactivity of HuMAb MN215 was found with the HIV-1 Universal consensus and the HIV-1 MN sequences. It also reacted with V3 domain peptides derived from the HIV-1 SC and SF2 strains. Since solid phase peptide ELISA values may not be considered to represent an adequate measurement of the relative affinity of an antibody for a given peptide, we also performed a peptide inhibition ELISA with the peptides showing reactivity with HuMAb MN215 (Fig. 3). HuMAb MN215 showed a relatively high affinity for the HIV-1 MN peptide and for the HIV-1 Universal V3 and HIV-1 SC peptides, but it bound with a much lower affinity to the HIV-1 SF2 peptide.

Minimum epitope recognized by HuMAb MN215

The minimum epitope recognized by HuMAb MN215 was determined by Pepscan analysis using 8 to 16 amino acid peptides, overlapping by all but one amino acid, spanning the HIV-1 MN V3 loop. The minimum epitope was defined as the shortest common sequence found in peptides reactive with HuMAb MN215. No reactivity could be found in this Pepscan analysis with overlapping peptides of 8 to 14 amino acids. The shortest peptide showing reactivity with HuMAb MN215 was a 15 amino acid peptide spanning the tip of the loop (GPGR) and amino acids at the C- and N-terminal side of this structure (Fig. 4*a*). A similar analysis was carried out with 7 to 13 amino acid peptides with the Dutch consensus sequence. The minimum epitope recognized by HuMAb MN215 with this V3 loop sequence proved to be 9 amino acids long and consisted of amino acids at the N-terminal side of the tip of the V3 loop only (Fig. 4*b*).

Individual amino acids involved in HuMAb MN215 binding

Differences between donor #658 and the Dutch consensus V3 domain sequences were introduced in a set of peptides based on the Dutch consensus sequence for Pepscan analysis. Substitutions S to G at position 13, T to A at position 28 and E to D at position 30, which were observed in donor #658 as compared to the Dutch consensus, did not influence the binding capacity of HuMAb MN215 (data not shown). The role of individual amino acids at both sides of the tip of the loop was determined by using peptide analogues which differed in only 1 amino acid from the peptide with the Dutch consensus sequence peptides in a separate Pepscan analysis (KSIHIGPGRAFYT) (Fig. 5).

Five single amino acid substitutions caused improved binding capacity of HuMAb MN215:

1) Substitution G to N at position 19 and R to K at position 20, which are both well tolerated at that position in a type II β turn (Wilmot & Thornton, 1988; Ghiara *et al.*, 1994).

2) F to L at position 23, which results in a smaller surface area and more rotational freedom.

3) Y to E and T to E at position 24, both resulting in a more negative charge on the C-terminal side of the tip of the loop.

Substitution of amino acids at the tip of the loop for amino acids with low frequency in known type II β turns were not well tolerated in the Pepscan with MN215 (20 P → L, 21 G → P, 21 G → A and 22 R → P; Wilmot & Thornton, 1988; Langedijk *et al.*, 1991). Reactivity of HuMAb MN215 with the V3 loop therefore seems to be dependent on the type II β turn at the tip of the loop (LaRosa *et al.*, 1990; Ghiara *et al.*, 1994).

A peptide was subsequently constructed in which all these mutations from the Dutch consensus sequence (KSIHIGPNKALEE) had been applied. HuMAb

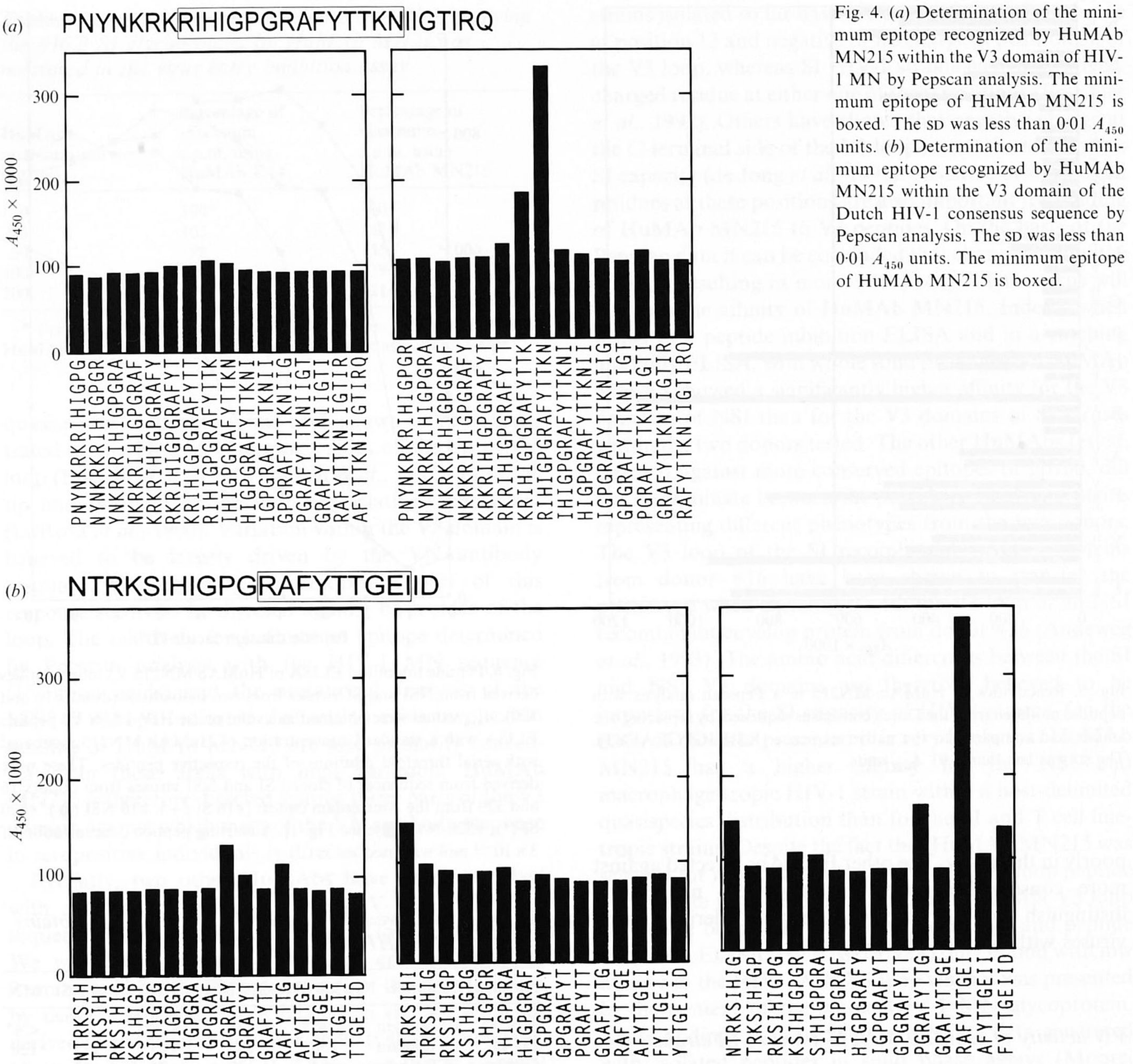


Fig. 4. (*a*) Determination of the minimum epitope recognized by HuMAb MN215 within the V3 domain of HIV-1 MN by Pepscan analysis. The minimum epitope of HuMAb MN215 is boxed. The SD was less than 0·01 A_{450} units. (*b*) Determination of the minimum epitope recognized by HuMAb MN215 within the V3 domain of the Dutch HIV-1 consensus sequence by Pepscan analysis. The SD was less than 0·01 A_{450} units. The minimum epitope of HuMAb MN215 is boxed.

MN215 failed to bind to this peptide in ELISA (data not shown).

Preferential reactivity of HuMAb MN215 with glycoprotein of NSI origin

The data obtained with the Pepscan analysis suggested a preferential reactivity of HuMAb MN215 for HIV-1 strains of the NSI phenotype. We therefore tested HuMAb MN215 in the peptide inhibition ELISA with V3 domain sequences derived from recombinant envelop proteins of cloned NSI and SI viruses from two donors, #16 and #320, of the Amsterdam cohort (Fig. 6). HuMAb MN215 showed a significantly higher relative affinity for the NSI- than for the SI-derived V3 peptides of both donors. We subsequently tested HuMAb MN215 together with three other HuMAbs directed against conserved epitopes on HIV-1 glycoproteins with the recombinant glycoproteins derived from the SI and NSI variants of donors #16 and #320. We also included glycoproteins from the HIV-1 MN isolate in this analysis. A significantly higher reactivity of HuMAb MN215 was found with the NSI glycoproteins as compared to the SI glycoproteins from both the donors (Table 1). HuMAb MN215 only recognized the HIV-1 MN (SI) glycoprotein

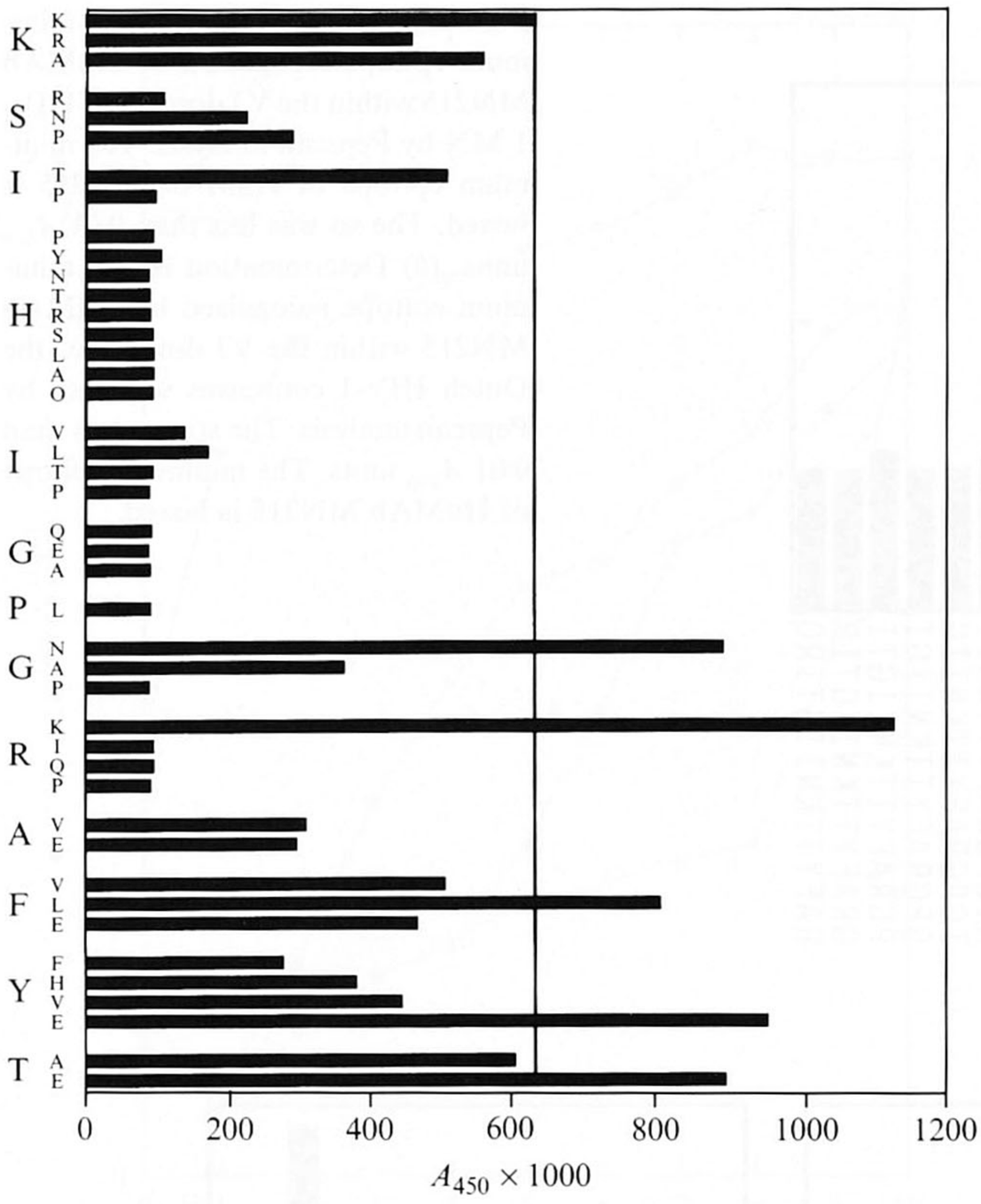


Fig. 5. Reactivities of HuMAb MN215 in a Pepscan analysis with peptides modified from the Dutch consensus sequence, by replacing one amino acid compared to the native sequence (KSIHIGPGRAFYT). The SD was less than 0·01 A_{450} units.

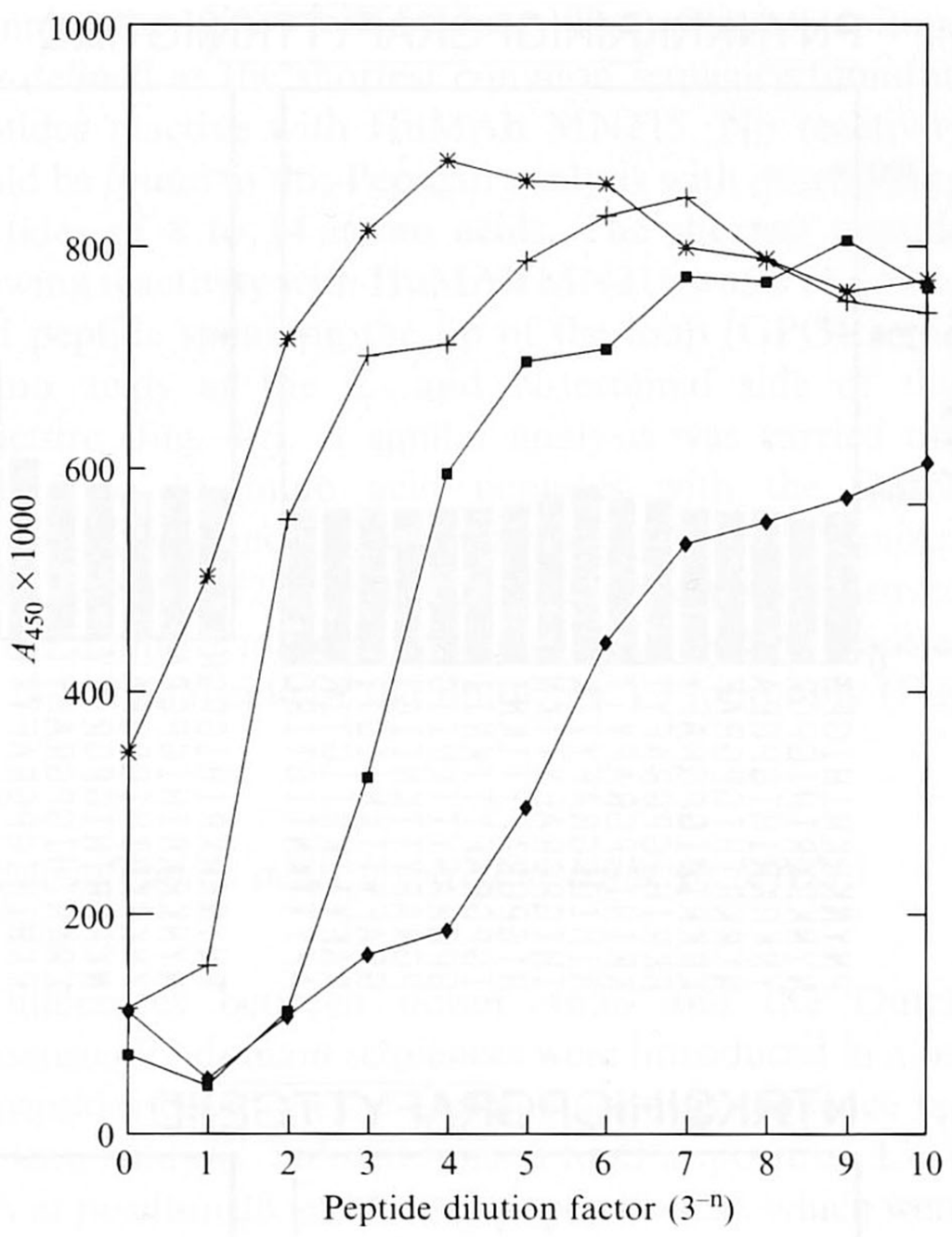


Fig. 6. Peptide inhibition ELISA of HuMAb MN215 V3 loop peptides derived from NSI and SI viruses from two individuals (donors 16 and 320). A_{450} values were obtained in a solid phase HIV-1 MN V3 peptide ELISA with a standard concentration of HuMAb MN215 incubated with serial threefold dilutions of the respective peptides. These were derived from sequences of cloned SI and NSI viruses from donors 16 and 320 from the Amsterdam cohort [#16 SI (+), #16 NSI (◆), #320 SI (*), #320 NSI (■); see Fig. 1]. A starting peptide concentration of 3×10^{-11} mol was used.

poorly in this assay. The other HuMAbs directed against more conserved epitopes on gp160 did not clearly distinguish between the glycoproteins derived from viruses with the two phenotypes.

VN activity of HuMAb MN215 towards SI strains

It has been shown by others that HIV-1 NSI strains are highly resistant to VN activity by human antibodies (Bou-Habib *et al.*, 1994). In accordance with these observations we failed to demonstrate inhibition of entry by chimeric HIV-1 carrying the recombinant 16.4 NSI glycoprotein in the virus entry inhibition assay (> 90% of c.p.m. without HuMAb at 200 μg/ml of HuMAb MN215). However, despite the low relative affinity of HuMAb MN215 for the SI HIV-1 glycoprotein from donor #16, it did inhibit entry of chimeric virus carrying the 16.2 SI glycoprotein, albeit at high concentrations (about 60 μg/ml for 50% inhibition and < 200 μg/ml needed for 90% inhibition). As expected HuMAb K14 failed to inhibit entry of these chimeric viruses (Table 2).

Table 1. *Relative affinity of HuMAbs for glycoproteins of SI and NSI HIV-1 strains*

HuMAb	Epitope	#320 SI	#320 NSI	#16 SI	#16 NSI	MN
MN215	V3 domain	< 4*†	48	< 4*	84	< 4*
257-D	V3 domain	250	250	125	125	128
GP13	CD4 bd	84	100	84	33	84
K14	gp41	42	10	84	55	42

* Dilution could not be determined because reactivity did not reach plateau value at the concentrations tested.

† Dilutions of the respective HuMAbs, starting at 1 μg/ml, at which 50% of the maximum binding was reached in the antibody capture ELISA with the cell lysates of rVV-infected HELA cells.

Discussion

We have described an HIV-1-neutralizing HuMAb (HuMAb MN215; IgG1) that proved to react with the V3 domain of a selection of subgroup B virus strains. This HuMAb displayed a higher affinity for NSI than for SI glycoproteins within the same host.

Variation in the V3 domain within the host-delimited

Table 2. *Inhibition of entry of chimeric HIV-1, carrying the #16.2 SI glycoprotein, by HuMAb MN215 as measured in the virus entry inhibition assay*

HuMAb concentration (μg/ml)	Percentage of maximum c.p.m. using HuMAb K14	Percentage of maximum c.p.m. using HuMAb MN215
0	100*	100
25	105	82·9
50	98	55·7
100	98	39·2
200	91	21·9

* Percentage from c.p.m. using 16.2 SI *env* gene without addition of HuMAb [(c.p.m. with HuMAb/c.p.m. without antibody) × 100].

quasispecies distribution has been shown to be concentrated at the N- and C-terminal sides of the tip of the loop (Epstein *et al.*, 1991; Kuiken *et al.*, 1992). Both the tip and the base of the loop are relatively conserved (LaRosa *et al.*, 1990). Variation within the V3 domain is believed to be largely driven by the VN antibody response. Consequently, an important part of this response seems to be directed against both sides of the loop. The relatively long minimum epitope determined by Pepscan analysis with the HIV-1 MN sequence (15 amino acids) and the mutational analysis of the MN215 epitope indicated that amino acids involved in binding of HuMAb MN215 are also primarily concentrated in these areas with high variation. HuMAb MN215 is therefore directed against those parts of the V3 domain against which most of the VN antibody response in seropositive individuals is directed.

Recently, two other HuMAbs have been described with a minimum epitope on the HIV-1 MN V3 loop sequence of 15 amino acids in length (Gorny *et al.*, 1993). We were able to narrow the core epitope down to 9 amino acids at the C-terminal side of the type II β turn by using a sequence more related to the V3 sequence derived from the virus of donor #658. It should, however, be stressed that the type II β turn and the N-terminal parts of the V3 loop also contribute to the optimum binding of the HuMAb.

Combining all amino acids giving improved binding of HuMAb MN215 in one peptide did not result in a peptide recognized by HuMAb MN215, indicating the importance of intramolecular interactions for optimum epitope presentation. Combining all the criteria for the 'best fitting' MN215-binding peptides with actually described V3 sequences of HIV-1 (LaRosa *et al.*, 1990) resulted in the following V3 sequence: KS/GIHIGPGKAFYTTGE/DI. This sequence exhibits a striking similarity with the conserved consensus V3 loop sequence of macrophage-tropic HIV-1 strains (Chesebro *et al.*, 1992). Previous reports have shown that all NSI strains isolated so far have the combination of no charge at position 13 and negative or no charge at position 30 of the V3 loop, whereas SI HIV-1 strains have a positively charged residue at either one of these positions (Fouchier *et al.*, 1992). Others have shown that negative charge at the C-terminal side of the V3 loop is associated with less SI capacity (de Jong *et al.*, 1992). Negative or uncharged residues at these positions are also important for binding of HuMAb MN215 to V3 peptides. On the basis of the Pepscan data it can be concluded that changes within the V3 loop resulting in more SI capacity for the virus will decrease the affinity of HuMAb MN215. Indeed, when tested in a peptide inhibition ELISA and in a catching antibody ELISA, with whole solid phase gp160, HuMAb MN215 showed a significantly higher affinity for the V3 domains of NSI than for the V3 domains of SI viruses within the two donors tested. The other HuMAbs tested, directed against more conserved epitopes of gp160, did not discriminate between the peptides and glycoproteins representing different phenotypes from the two donors. The V3 loop of the SI recombinant envelop proteins from donor #16 have been shown to transfer the phenotype when exchanged with the V3 loop of an NSI recombinant envelop protein from donor #16 (Andeweg *et al.*, 1993). The amino acid differences between the SI and NSI V3 domains are therefore believed to be important for the SI capacity of HIV-1 strains. On the basis of the data presented it is very likely that HuMAb MN215 has a higher affinity for the NSI and macrophage-tropic HIV-1 strain within a host-delimited quasispecies distribution than for the SI and T cell line-tropic strains. Despite the fact that HuMAb MN215 was selected for reactivity with a HIV-1 MN V3 loop peptide and despite its high affinity for the HIV-1 MN V3 loop peptide in both solid phase peptide ELISA and peptide inhibition ELISA, HuMAb MN215 only bound with low affinity to the HIV-1 MN V3 loop when it was presented in the context of the whole HIV-1 MN glycoprotein. These findings indicate that antigenicity data generated with isolated peptides in solid phase assays (Moore, 1993) and also with isolated peptides in solution may not be representative of the antigenicity of these structures in their natural configuration. It has been shown that macrophage-tropic HIV-1 strains need a certain conformation of their V3 loop (Innocenti-Francillard *et al.*, 1994), which is apparently recognized by HuMAb MN215. This may explain why the 'suboptimum' V3 sequence of donor #320 NSI, which is not recognized by HuMAb MN215 in Pepscan analysis (data not shown), still results in a glycoprotein that is more efficiently recognized than the #16 SI or the #320 SI glycoproteins. The low relative affinity of HuMAb MN215 for the HIV-1 MN glycoprotein is in accordance with the low reactivity in FACScan analysis of this antibody observed

with HIV-1 MN-infected SupT1 cells (unpublished results) and the relatively low VN capacity of this antibody towards HIV-1 MN and SF2 (A. McKnight, personal communication). We also showed that HuMAb MN215 inhibited entry of a chimeric HIV-1 strain carrying the recombinant 16.2 SI HIV-1 glycoprotein, albeit very inefficiently. The CD4-binding domain (CD4 bd)-specific HuMAb GP13, which neutralizes T cell line-adapted HIV-1 strains (Schutten *et al.*, 1993), did not neutralize the 16.2 SI chimeric virus at the concentrations tested (data not shown) although this antibody has a significantly higher relative affinity for the 16.2 SI glycoprotein than HuMAb MN215. This stresses the relatively high VN capacity of antibodies directed against the V3 domain. It was unexpected that HuMAb MN215 did not inhibit entry of a chimeric virus carrying the 16.4 NSI glycoprotein, which it preferentially recognizes. However, an affinity-purified immunoglobulin preparation from seropositive individuals (HIVIG; Prince *et al.*, 1991) also failed to do so (data not shown). The inability of HuMAb MN215 to neutralize an NSI virus under conditions at which it does neutralize an SI virus, which it recognizes with lower affinity, should therefore be considered in the light of general difficulties encountered with the neutralization of non-T cell line-adapted NSI HIV-1 strains (Bou-Habib *et al.*, 1994).

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