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Synergistic Neutralization of Human Immunodeficiency Virus Type 1 by a Chimpanzee Monoclonal Antibody against the V2 Domain of gp120 in Combination with Monoclonal Antibodies against the V3 Loop and the CD4-Binding Site

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Synergistic neutralization of human immunodeficiency virus type 1 (HIV-1) was observed in studies using a chimpanzee anti-V2 monoclonal antibody (MAb), C108G, in combination with anti-V3 loop and anti-CD4 binding-site (bs) MAbs of different epitope specificities. C108G paired with either of two anti-V3 loop MAbs or either of two anti-CD4 bs MAbs synergistically neutralized both the uncloned IIIB and clonal HXB2 strains of virus in H9 target cells. Synergism was quantitated by calculation of combination indices. Significant synergy with a given MAb pair was seen over a range of MAb ratios, with the optimal effect centering around the ratio at which the MAbs were equipotent for a given HIV-1 strain (on the basis of the 50% neutralization titer). In preliminary experiments with monocytotropic strains of HIV-1 in peripheral blood mononuclear cell targets, significant synergism was also observed between anti-V2–anti-V3 and anti-V2–anti-CD4 bs MAb pairs. Synergism by all MAb pairs tested was greater against heterogeneous isolates of HIV-1 (IIIB and Ba-L) than against clonal isolates (HXB2 and NLHXADA), suggesting that strain broadening may be a component of the synergism observed against the heterogeneous isolates. In addition, conformational changes in gp120 upon binding of one or both MAbs may result in increased affinity or exposure of the epitope of one or both MAbs. Finally, a three-MAb combination of C108G, an anti-V3 MAb, and an anti-CD4 bs MAb was more effective in neutralizing the HXB2 strain of HIV-1 than any of the three two-MAb combinations within this trio, as determined by the dose reduction indices of each MAb required to achieve a given level of neutralization. This is the first report of synergistic neutralization of HIV-1 by a three-MAb combination composed of MAbs directed against the three major neutralization epitope clusters in gp120. Implications for vaccine design and for immunoprophylaxis and immunotherapy with a combination of MAbs are discussed.

Synergistic neutralization of human immunodeficiency virus type 1 (HIV-1) by antibodies or other ligands directed against different epitopes of HIV-1 envelope (Env) has been studied in vitro by several groups over the past few years (1, 2, 4, 18, 21, 22, 24, 25, 33, 38, 40), using primarily monoclonal antibodies (MAbs) of defined epitope specificity and laboratory-adapted strains of HIV-1. The synergistic interactions observed in vitro could be significant in vivo, provided that the antibodies synergistically neutralize clinical isolates of HIV-1 and that such antibodies of two or more different specificities are present simultaneously at appropriate ratios in vivo. Such antibodies could be elicited by vaccination or could be passively administered in the form of a MAb cocktail. Such synergism is likely to be desirable because it often involves broadening of the viral strain specificity of the MAb combination compared with that of one or both MAbs alone (2, 22, 24), is likely to represent a greater challenge for the virus to evolve neutralization escape mutants, and, by definition, involves higher levels of viral neutralization at a given dose of the MAb combination than of either MAb alone.

Four major neutralization epitope clusters have been found in HIV-1 Env of laboratory-adapted strains. These are the V3 loop (reviewed in references 27 and 41), CD4 binding site (bs) (reviewed in reference 41), and V2 domain (reviewed in references 19 and 43) in gp120 and the 2F5 MAb-defined site (30) in gp41. Some epitopes within each of these neutralization epitope clusters appear to be exposed at least partially on Env of primary and/or monocytotropic isolates (3, 8, 9, 13); the latter appear to be the major HIV-1 strains transmitted during infection (47) and present during the asymptomatic phase of infection (34). Synergistic neutralization has been studied most extensively between anti-V3 and anti-CD4 bs MAbs and ligands (1, 2, 4, 22, 24, 25, 33, 38, 40); this is often a highly synergistic interaction but does not occur between every anti-V3–anti-CD4 bs MAb pair studied. Synergism between soluble CD4 and anti-V3 MAbs has been observed against a monocytotropic strain of HIV-1 grown in peripheral blood mononuclear cells (PBMC) (33) as well as for laboratory-adapted strains grown in T-cell lines (1, 2, 4, 22, 24, 33, 38, 40). In addition, synergism between two anti-CD4 bs MAbs differing in specificity and synergism between anti-CD4 bs MAbs and a nonneutralizing MAb against the C terminus of gp120 have been reported (21). In an HIV-1 Env-mediated cell fusion assay which contrasts with the cell-free virus neutralization assays used by other investigators studying synergistic neutralization of HIV-1, Allaway et al. (1) observed synergistic interteractions between the anti-gp41 neutralizing MAb, 2F5, and anti-CD4 bs MAbs and between anti-V3 and anti-CD4 bs MAbs.

The interaction of anti-V2 MAbs with MAbs directed against other Env epitopes in HIV-1 neutralization had not

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been explored until the current study. In this report, we document the synergistic neutralization of various HIV-1 strains by anti-V2 MAb C108G (42, 43, 45) in combination with anti-V3 MAbs and/or anti-CD4 bs MAbs.

MATERIALS AND METHODS

HIV-1 isolates. The IIIB and HXB2 stocks of HIV-1 were prepared in H9 cells. The stock of HXB2, which is a molecular clone of the IIIB strain, was prepared by transfecting DNA into H9 cells by the DEAE method, using a CellPhect transfection kit (Pharmacia, Piscataway, N.J.), followed by a few further passages in H9 cells to generate a high-titer stock. The Ba-L (12) stock was prepared in phytohemagglutinin-activated PBMC. The infectious molecular clone of NLHXADA (44) was transfected into 293 (human adenocarcinoma) cells, and supernatant containing virus was harvested after 72 h; this supernatant constituted the NLHXADA stock.

MAbs. Protein A-purified preparations of human MAbs 1125H (39) and 5145A (31), which are directed against distinct epitopes overlapping the CD4 bs of HIV-1 gp120, chimpanzee MAb C108G (42, 43, 45), whose epitope maps to an *N*-glycan-dependent epitope in the V2 domain of gp120, and chimpanzee MAb C311E (41a), against the V3 loop of gp120, were used. MAb C311E was isolated and characterized essentially as described for other human and chimpanzee anti-Env MAbs (39, 43); these results will be published elsewhere. The anti-V3 loop mouse MAb 0.5β (23) was used in the form of ascites fluid. Control neutralization experiments done with the latter against the MN strain of HIV-1 showed no neutralization of this strain, indicating that there were no nonspecific neutralizing components in the ascites fluid preparation.

Neutralization assays. In the standard assay involving viral infection of H9 cells, various concentrations of MAbs or MAb combinations were preincubated as described previously (35, 39) with approximately 2×10^4 infectious units of IIIB or 6×10^3 to 8×10^4 infectious units of HXB2. These MAb-virus combinations were then incubated with H9 cells for 24 h for the IIIB isolate or 48 h for the HXB2 virus. Following this incubation, numbers of infected cells in control wells without MAbs and in experimental wells were assessed by immunofluorescence, and percent neutralization was calculated as detailed elsewhere (35, 39).

A modification of this assay (15) was used to assess neutralization of monocytotropic isolates infecting phytohemagglutinin-activated PBMC derived from seronegative donors. Virus stocks were preincubated with various concentrations of MAbs or MAb combinations as in the standard assay (35, 39) and added to 5×10^6 activated PBMC in a total volume of 400 μl in Falcon 48-well tissue culture plates. The cells were then incubated until 2 to 5% of the cells were infected in the control wells lacking MAbs, typically 72 to 96 h postinfection. To assess the percentage of cells infected, viral antigens in the fixed cells were detected by a biotinylated preparation of human HIV immunoglobulin at 0.5 mg/ml followed by a 1/50 dilution of fluorescein isothiocyanate-conjugated streptavidin (Zymed Laboratories, Inc., South San Francisco, Calif.).

Data analysis. The dose-effect analysis computer program of Chou and Chou (5) (Biosoft, Cambridge, United Kingdom) was used to analyze the data as detailed elsewhere (40). Briefly, this program fits the neutralization data obtained with each MAb and MAb combination to the median-effect equation and then calculates a set of combination index (CI) values over the complete range of neutralization levels (>0 to 100% neutralization). The CI values are generated by the program on the basis of mutually exclusive (for MAbs having similar modes of action) and mutually nonexclusive (for MAbs having different modes of action or acting independently) assumptions. For MAbs having different modes of action or acting independently, α equals 1, whereas for MAbs having the same or similar modes of action, α equals 0 (7, 40). We previously argued that MAbs against different epitopes of HIV-1 envelope which do not compete with one another for binding are acting independently and that the mutually nonexclusive assumption should be used for analyses of neutralization by combinations of such MAbs. However, Kennedy et al. (18) argued that the actions of neutralizing MAbs against different epitopes of HIV-1 envelope are mutually exclusive on the basis of the assumption that all MAbs are acting at the same step in blocking virus infection, i.e., viral entry. Since these assumptions are a matter of interpretation and cannot be objectively verified, we have chosen to present CI values calculated on the basis of both the mutually exclusive and nonexclusive assumptions for all of the two-MAb combination experiments presented in this report. For three-MAb combinations, the dose-effect analysis program calculates only CI values based on the mutually exclusive assumption.

To facilitate visualization of synergistic neutralization effects, we have compared the computer-generated best-fit curve of the neutralization data obtained by using a given MAb combination with a predicted neutralization curve which would be expected for that MAb combination if the MAbs in the combination acted additively. The latter curve was generated by setting CI to 1 and solving the equation CI (at a given percent neutralization) = $D_{A,mix}/D_{A,alone} + D_{B,mix}/D_{B,alone}$ (mutually exclusive assumption) (40) for the dose (D), or concentration, of the combination of MAbs ($D_{A,mix} + D_{B,mix}$) required to attain a given percent neutralization, using the $D_{A,alone}$ and $D_{B,alone}$ values at a given percent neutralization generated by the computer program in obtaining the best fit of the neutralization curves of each MAb alone. These calculations were possible because of known ratios of MAbs A and B in the MAb combinations, i.e., known relationships between $D_{A,mix}$ and $D_{B,mix}$.

The linear coefficient, r, indicates the goodness of fit of the data to the median-effect equation, where a value of 1 indicates a perfect fit. The dose reduction index is the concentration of either of the MAbs alone required to effect a given level of neutralization divided by the concentration of that MAb in combination required to effect that level of neutralization.

RESULTS

Synergism of anti-V2 MAb C108G with anti-V3 MAbs in HIV-1 neutralization. Previous reports described the glycan-dependent epitope in the V2 domain of gp120 recognized by chimpanzee MAb C108G (43, 45) and the latter's very potent neutralization of the IIIB and HXB2 strains of HIV-1 (43) as assessed in a fluorescent focus assay. Specifically, C108G neutralized 50% of IIIB virions at approximately 15 ng/ml and 50% of HXB2 virions at approximately 3 ng/ml in this assay. In this study, we used a new anti-V3 chimpanzee MAb, C311E. Preliminary characterization of this MAb indicates that it is broadly strain reactive and maps to an epitope involving residues RKRIHIGP (Los Alamos numbers 309 to 316 in the MN strain) and perhaps additional flanking amino acids (41a). C311E neutralized 50% of IIIB or HXB2 virions at approximately 10 ng/ml in the fluorescent focus assay (data not shown) and is therefore comparable in potency to C108G in neutralizing infection of H9 cells by these two strains passaged in H9 cells.

Neutralization of IIIB and HXB2 on H9 target cells was evaluated in several experiments in which approximately equipotent amounts (1:1 ratio) of C108G and C311E were combined and the results were compared with those for each MAb alone (data not shown). Average r values and CI values were calculated for these experiments by the Chou-Talalay method (5, 7) and are shown for selected medium to high (50 to 95%) neutralization levels in Table 1. Synergism is indicated by a CI of <1; the lower the CI values, the greater the synergism observed. The results show that significant synergism was observed against the IIIB strain with this pair of MAbs (CI = 0.35 to 0.57), while a lower but reproducible level of synergism was observed against the clonal HXB2 virus; this was most evident at medium levels of neutralization (CI = 0.61 to 0.81 at 50 to 75% neutralization). To address whether synergism of the C108G-C311E combination against HXB2 was being underestimated because of the ratio of MAbs used, mixtures of C108G and C311E combined at different ratios were tested against this isolate (data not shown). A 1:5 ratio of C108G to C311E resulted in synergism (average CI from 50 to 95% neutralization = 0.74) similar to or slightly greater than that for the 1:1 ratio (Table 1), whereas 25:1 and 1:25 ratios of C108G to C311E were essentially additive. Thus, use of approximately equipotent amounts of C108G and C311E, i.e., 1:1 to 1:5 ratios, yielded the best synergism.

Recently, we have obtained preliminary evidence that the synergism between C108G and C311E extends to certain monocytotropic strains of HIV-1 passaged in PBMC. Figure 1A shows representative neutralization data for C108G, C311E, and a 1:1 C108G-C311E combination versus the Ba-L isolate (12); these results were obtained by using a modification of the fluorescent focus assay (15) involving PBMC rather than H9 cells as targets. The neutralization curve obtained for the MAb combination is shifted to the left of the curves for C108G alone and C311E alone, indicating a synergistic interaction between two MAbs similar in potency as seen in previous studies (40). Another means of visualizing synergism between these MAbs is shown in Fig. 1B, in which the computer-generated best-fit curve of the neutralization data for the MAb

TABLE 1. Average CI and *r* values calculated[a] from data obtained in assays of neutralization of HIV-1 by two-MAb combinations[a]

MAb(s) used	Ratio	Strain of HIV-1 used	*r*[b]	CI[b] at % neutralization of:			
				50	75	90	95
C108G			0.93 ± 0.01				
C311E			0.97 ± 0.00				
C108G + C311E	1:1	IIIB	0.97 ± 0.00	0.35 ± 0.09[c]	0.38 ± 0.02	0.47 ± 0.11	0.57 ± 0.24
				0.37 ± 0.09[d]	0.38 ± 0.01	0.47 ± 0.11	0.57 ± 0.24
C108G			0.97 ± 0.00				
C311E			0.97 ± 0.01				
C108G + C311E	1:1	HXB2	0.95 ± 0.02	0.61 ± 0.14	0.70 ± 0.17	0.81 ± 0.21	0.89 ± 0.25
				0.70 ± 0.19	0.81 ± 0.24	0.95 ± 0.32	1.07 ± 0.40
C108G			0.90 ± 0.00				
C311E			0.99 ± 0.00				
C108G + C311E	1:1	Ba-L	0.99 ± 0.00	0.22 ± 0.00	0.16 ± 0.00	0.15 ± 0.00	0.15 ± 0.00
				0.23 ± 0.00	0.17 ± 0.00	0.15 ± 0.00	0.16 ± 0.00
C108G			0.91 ± 0.07				
0.5β			0.92 ± 0.04				
C108G + 0.5β	1:25	IIIB	0.96 ± 0.01	0.25 ± 0.07	0.16 ± 0.11	0.17 ± 0.17	0.19 ± 0.29
				0.26 ± 0.07	0.16 ± 0.11	0.17 ± 0.18	0.19 ± 0.22
C108G			0.96 ± 0.02				
0.5β			0.98 ± 0.01				
C108G + 0.5β	1:25	HXB2	0.96 ± 0.03	0.37 ± 0.01	0.44 ± 0.09	0.56 ± 0.06	0.69 ± 0.15
				0.38 ± 0.07	0.48 ± 0.08	0.62 ± 0.13	0.75 ± 0.16
C108G			0.84 ± 0.00				
5145A			0.99 ± 0.02				
C108G + 5145A	1:25	IIIB	0.98 ± 0.01	0.25 ± 0.01	0.20 ± 0.08	0.23 ± 0.11	0.26 ± 0.13
				0.27 ± 0.02	0.20 ± 0.08	0.23 ± 0.11	0.26 ± 0.12
C108G			0.96 ± 0.01				
5145A			0.97 ± 0.02				
C108G + 5145A	1:25	HXB2	0.94 ± 0.04	0.60 ± 0.13	0.60 ± 0.02	0.59 ± 0.11	0.56 ± 0.23
				0.71 ± 0.21	0.64 ± 0.21	0.63 ± 0.23	0.63 ± 0.29
C108G			0.89 ± 0.08				
1125H			0.98 ± 0.03				
C108G + 1125H	1:25	IIIB	0.98 ± 0.00	0.16 ± 0.03	0.15 ± 0.06	0.18 ± 0.12	0.21 ± 0.17
				0.16 ± 0.04	0.15 ± 0.06	0.18 ± 0.12	0.21 ± 0.17
C108G			0.96 ± 0.07				
1125H			0.98 ± 0.04				
C108G + 1125H	1:25	HXB2	0.95 ± 0.03	0.44 ± 0.23	0.48 ± 0.16	0.54 ± 0.07	0.62 ± 0.10
				0.46 ± 0.23	0.49 ± 0.16	0.55 ± 0.07	0.63 ± 0.11

[a] CI and *r* values were calculated by using the dose-effect analysis computer program (5), where CIs of <1, =1, and >1 indicate synergism, additivity, and antagonism, respectively (7).
[b] Average ± standard deviation obtained from two to five independent experiments.
[c] Calculated by using the mutually exclusive assumption as in references 7 and 40.
[d] Calculated by using the mutually nonexclusive assumption as in references 7 and 40.

combination is compared with the theoretical curve that depicts expected results if the combination acted additively. This analysis shows that the experimental curve is shifted to the left of the predicted additive curve at significant (>25%) neutralization levels. These analyses are corroborated by CI values ranging from 0.15 to 0.23 over 50 to 95% neutralization of Ba-L as seen in Table 1. This synergism is even greater than that seen with this MAb pair against either IIIB or HXB2 in H9 target cells (Table 1).

Figure 2 and Table 1 show results of combining C108G with another anti-V3 MAb, mouse MAb 0.5β (23, 36), in neutralization of the IIIB and HXB2 strains. The epitopes of 0.5β and C311E are distinct and fall predominately on different sides of the V3 loop, though they may overlap at the tip of the loop (36, 41a). Since C108G was previously found to be approximately 30-fold more potent than 0.5β in neutralization of IIIB (43), the MAbs were combined at a 1:25 ratio of C108G to 0.5β in these experiments. Figure 2 shows representative data for neutralization of IIIB (Fig. 2A) and HXB2 (Fig. 2B); in each panel, the neutralization curve obtained for the MAb combination actually falls between the curves for C108G alone and 0.5β alone. Thus, when combinations of MAbs that are substantially different in potency are used in such assays, it is difficult, if not impossible, to assess the interaction between them by inspection of the raw neutralization data. However, in the computer analyses shown in Fig. 2C and D, for this MAb combination, the experimental curves are shifted to the left of the predicted additive curves at significant (>25%) neutralization levels. As the CI values in Table 1 corroborate, this is a clear means of visualizing synergism even when visualization from the rough neutralization data is difficult. Interestingly, this analysis (Fig. 2C and D) and the CI values shown in Table 1 for the C108G-0.5β combination indicate that this combination is more synergistic than the C108G-C311E combination against IIIB and HXB2. Greater synergism was observed against IIIB than against HXB2 with both pairs of MAbs (Table 1). Ratios of C108G to 0.5β ranging from 1:10 to 1:75 synergistically neutralized IIIB and HXB2 at levels approxi-

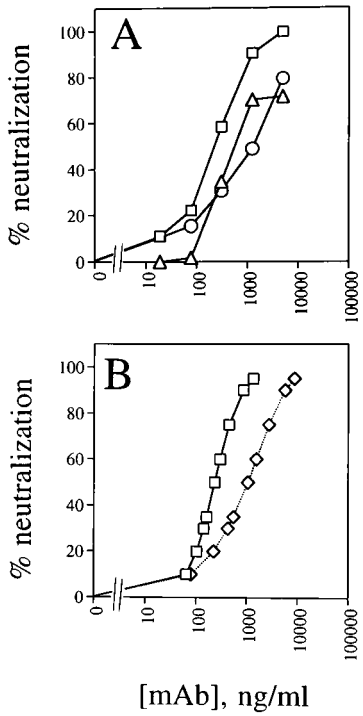

FIG. 1. Neutralization of Ba-L infection of PBMC by anti-V2 MAb C108G and anti-V3 MAb C311E used individually or in a 1:1 ratio and analysis of results based on the Chou-Talalay method (7), using the dose-effect analysis computer program (5). (A) Neutralization of the Ba-L strain obtained with C108G (△), C311E (○), or a 1:1 ratio of C108G and C311E (□). The total amount of the mixture is plotted; i.e., C108G and C311E are each present in the mixture at one-half the plotted concentration. (B) The computer-generated best fit (□) of the neutralization data obtained with the MAb combination (C108G plus C311E, 1:1 ratio) versus Ba-L as shown in panel A to the median-effect equation compared with a theoretically calculated curve (◇) obtained for the same MAb combination, assuming its interaction to be additive in neutralization. See Materials and Methods for details.

mating those seen with the 1:25 ratio, and synergism against IIIB was always greater than that observed against HXB2 at a given ratio (data not shown). A 1:125 ratio of C108G to 0.5β against HXB2 yielded additive results (data not shown).

Synergism of anti-V2 MAb C108G with anti-CD4 bs MAbs in HIV-1 neutralization. To assess the interaction of C108G with anti-CD4 bs MAbs, the former was paired with human MAbs 5145A (31) and 1125H (37, 39), which are directed against distinct but overlapping epitopes. Results of neutralization of IIIB and HXB2 by these MAbs and MAb pairs are shown in Fig. 3 and Table 1. Figure 3 shows representative neutralization data for C108G, 5145A, and a 1:25 C108G-5145A combination against IIIB (Fig. 3A) and HXB2 (Fig. 3B); in each panel, the neutralization curve obtained for the MAb combination actually falls between the curves for C108G alone and 5145A alone. Nevertheless, the computer analyses shown in Fig. 3C and D clearly indicate that these MAbs act synergistically in neutralizing the IIIB and HXB2 strains. As was previously observed for the C108G-C311E and C108G-0.5β combinations (Table 1; Fig. 2C and D), the C108G-5145A combination was more synergistic against IIIB than against HXB2 (Table 1; Fig. 3C and D). The magnitude of the synergy obtained with C108G plus 5145A against IIIB is similar to that obtained with C108G plus 0.5β against this strain (compare Fig. 2C and 3C and CI values in Table 1). Results obtained against IIIB and HXB2 with the C108G-1125H pair at a 1:25 ratio were very similar to those seen with the C108G-5145A pair at this ratio (Table 1). Ratios of C108G to 1125H ranging from 1:5 to 1:60 synergistically neutralized IIIB at levels approximating those seen with the 1:25 ratio, while a 1:125 ratio of these MAbs was clearly less synergistic than the former ratios (data not shown).

Recent preliminary evidence suggests that the synergism between C108G and anti-CD4 bs MAbs extends to certain monocytotropic strains of HIV-1 passaged in PBMC (14a). In neutralization of the Ba-L isolate by a 1:1 combination of C108G and an anti-CD4 bs MAb, immunoglobulin IgG1b12 (3), in a modification of the fluorescent focus assay using PBMC as targets (data not shown), we observed CI values ranging from 0.15 to 0.54 over 50 to 95% neutralization. Further studies of neutralization by such MAb combinations with PBMC as targets are in progress.

Synergism among three MAbs, anti-V2 MAb C108G, anti-V3 MAb 0.5β, and anti-CD4 bs MAb 1125H, in HIV-1 neutralization. As shown above, C108G synergistically neutralized HIV-1 when paired with either anti-V3 loop MAbs or anti-CD4 bs MAbs. We (40) and others (1, 2, 4, 22, 24, 33, 38) previously showed that certain pairs of anti-V3 loop and anti-CD4 bs MAbs synergistically neutralized HIV-1 as well. Therefore, it

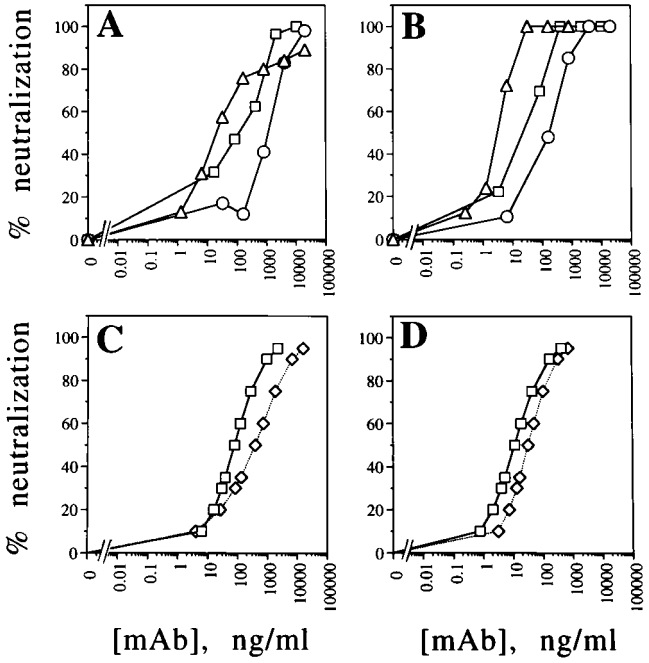

FIG. 2. Neutralization of IIIB and HXB2 infection of H9 cells by anti-V2 MAb C108G and anti-V3 MAb 0.5β used individually or in a 1:25 ratio and analysis of results based on the Chou-Talalay method (7), using the dose-effect analysis computer program (5). (A) Neutralization of the IIIB strain obtained with C108G (△), 0.5β (○), or a 1:25 ratio of C108G and 0.5β (□). The total amount of the mixture is plotted; i.e., C108G is present in the mixture at 1/26 times the plotted concentration, and 0.5β is present in the mixture at 25/26 times the plotted concentration. (B) Neutralization of the HXB2 clone obtained with C108G (△), 0.5β (○), or a 1:25 ratio of C108G and 0.5β (□). The total amount of the mixture is plotted as described for panel A. (C) The computer-generated best fit (□) of the neutralization data obtained with the MAb combination (C108G plus 0.5β, 1:25 ratio) against IIIB (shown in panel A) to the median-effect equation compared with a theoretically calculated curve (◇) obtained for the same MAb combination, assuming its interaction to be additive in neutralization. (D) The computer-generated best fit (□) of the neutralization data obtained with the MAb combination (C108G plus 0.5β, 1:25 ratio) against HXB2 as shown in panel B to the median-effect equation compared with a theoretically calculated curve (◇) obtained for the same MAb combination, assuming its interaction to be additive in neutralization.

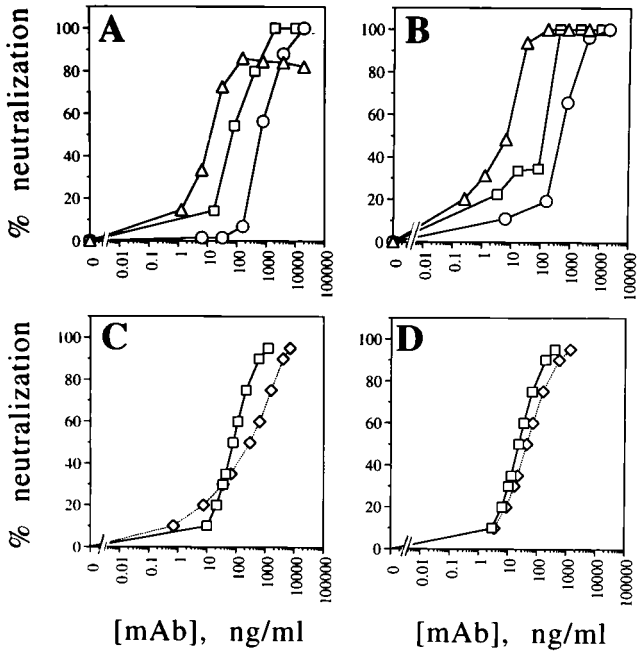

FIG. 3. Neutralization of IIIB and HXB2 infection of H9 cells by anti-V2 MAb C108G and anti-CD4 bs MAb 5145A used individually or in a 1:25 ratio and analysis of results based on the Chou-Talalay method (7), using the dose-effect analysis computer program (5). (A) Neutralization of the IIIB strain obtained with C108G (△), 5145A (○), or a 1:25 ratio of C108G and 5145A (□). The total amount of the mixture is plotted; i.e., C108G is present in the mixture at 1/26 times the plotted concentration, and 5145A is present in the mixture at 25/26 times the plotted concentration. (B) Neutralization of the HXB2 clone obtained with C108G (△), 5145A (○), or a 1:25 ratio of C108G and 5145A (□). The total amount of the mixture is plotted as described for panel A. (C) The computer-generated best fit (□) of the neutralization data obtained with the MAb combination (C108G plus 5145A, 1:25 ratio) against IIIB as shown in panel A to the median-effect equation compared with a theoretically calculated curve (◇) obtained for the same MAb combination, assuming its interaction to be additive in neutralization. (D) The computer-generated best fit (□) of the neutralization data obtained with the MAb combination (C108G plus 5145A, 1:25 ratio) against HXB2 as shown in panel B to the median-effect equation compared with a theoretically calculated curve (◇) obtained for the same MAb combination, assuming its interaction to be additive in neutralization.

was of interest to see whether the synergistic interactions between these three types of two-MAb combinations (anti-V2–anti-V3, anti-V2–anti-CD4 bs, and anti-V3–anti-CD4 bs) would be retained and/or enhanced in a three-MAb combination. Table 2 shows various parameters calculated by using the dose-effect analysis computer program from such a representative neutralization experiment against HXB2. The anti-V3 loop MAb, 0.5β, and the anti-CD4 bs MAb, 1125H, were used at a 1:1 ratio to each other when paired and when used with C108G in the three-MAb combination. Both 0.5β and 1125H were used at a 25-fold excess over C108G when each was paired with C108G and in the three-MAb combination. Focusing first on the CI values, we see that those for the three-MAb combination were somewhat better (i.e., lower) overall than those for the most synergistic two-MAb combination, i.e., C108G plus 1125H, and significantly better than those for the other two-MAb combinations. Therefore, synergism among the three MAbs is at least as great as, if not greater than, that observed for any of the three two-MAb combinations. Furthermore, the dose reduction indices were significantly higher for the three-MAb combination than any of the two-MAb combinations, indicating that the three-MAb combination has a more beneficial effect than any of the two-MAb combinations (20).

DISCUSSION

Results presented here show that anti-V2 MAb C108G synergistically neutralizes both laboratory-adapted and monocytotropic strains of HIV-1 when paired with anti-V3 or anti-CD4 bs MAbs of different epitope specificities. Optimal synergy with a given MAb pair was seen at a range of MAb ratios centering around the ratio at which the MAbs were equipotent for a given HIV-1 strain (on the basis of the 50% neutralization titer). In addition, a three-MAb combination of C108G, an anti-V3 MAb, and an anti-CD4 bs MAb was more effective in neutralizing the HXB2 strain of HIV-1 than any of the three two-MAb combinations within this trio. Further studies will assess whether this three-way synergism extends to primary and/or monocytotropic isolates; the synergism of the relevant two-MAb combinations against such isolates suggests that it will.

The mechanism of these synergistic interactions remains to be defined, but the following points appear relevant. First, we observed that synergism with all combinations of C108G–anti-V3 and C108G–anti-CD4 bs MAbs tested was higher against the heterogeneous IIIB and Ba-L isolates than against the clonal isolate HXB2. In preliminary neutralization studies using the clonal, monocytotropic NLHXADA isolate (44) and PBMC as targets, we have also observed lower levels of synergism with a given MAb pair against this isolate than against IIIB and Ba-L (14a). This finding suggests that a component of the synergistic interaction of MAbs against the heterogeneous isolates is strain broadening, i.e., the phenomenon of a MAb acquiring the ability to significantly neutralize, in the presence of another MAb, certain clones of virus which it does not significantly neutralize alone. Previous studies by other investigators have documented this phenomenon for certain anti-V3 MAbs in the presence of soluble CD4 (22, 24). Nevertheless, the fact that moderate synergism between C108G and the anti-V3 and anti-CD4 bs MAbs is seen against the clonal HXB2 and NLHXADA isolates suggests that conformational changes in gp120 upon binding of one or both MAbs may result in increased affinity or exposure of the epitope of one or both MAbs, as has previously been found for certain pairs of anti-V3–anti-CD4 bs MAbs (32, 46).

Interestingly, Davis et al. (10) previously reported that antisera separately raised against V2 and V3 peptides gave a higher neutralization index when acting together than when used alone. While synergism was not clearly documented in this study, the results are consistent with findings presented here. Also, Moore et al. (28) noted that some HIV-1-positive sera enhanced the binding of anti-V2 MAbs to monomeric and oligomeric gp120 and suggested that the serum antibodies responsible for this effect either were of high affinity or were present at relatively high concentrations. To address the specificity of these serum antibodies, various MAbs of known epitope specificity were tested for the ability to enhance anti-V2 MAb binding to HIV-1 Env. Certain anti-CD4 bs and anti-C4 domain MAbs were able to perform this function (28). In a more recent study, it was shown that certain anti-V2–anti-CD4 bs and anti-V2–anti-V3 MAb pairs uni- or bidirectionally enhanced each other's binding to monomeric gp120 (29). These findings are consistent with our observations of synergistic neutralization of HIV-1 by anti-V2 MAb C108G in combination with anti-CD4 bs and/or anti-V3 MAbs and suggest, as discussed above, that induced changes in gp120 conformation are partially responsible for this synergism.

Though present in high concentrations in seropositive serum, most human anti-CD4 bs MAbs characterized to date (IgG1b12 being an exception) appear to have poor neutralizing

TABLE 2. CI and dose reduction indices calculated[a] from representative neutralization data obtained by using two- and three-MAb combinations against HIV-1_{HXB2}

Concn(s) (μg/ml) of MAb(s)[b] required to attain a given % neutralization			% Neutralization	CI	DRI		
C108G	1125H	0.5β			C108G	1125H	0.5β
0.293			95				
0.114			90				
0.028			75				
0.007			50				
	501.09		95				
	121.58		90				
	15.16		75				
	1.89		50				
		8.81	95				
		3.90	90				
		1.18	75				
		0.36	50				
0.068 +	1.69		95	0.23	4.32	296.10	
0.031 +	0.77		90	0.28	3.71	158.62	
0.010 +	0.24		75	0.35	3.00	63.36	
0.003 +	0.08		50	0.46	2.36	25.30	
	5.96 +	5.96	95	0.69		84.14	1.48
	2.93 +	2.93	90	0.77		41.54	1.33
	1.03 +	1.03	75	0.94		14.72	1.14
	0.36 +	0.36	50	1.21		5.20	0.98
0.055 +		1.38	95	0.35	5.30		6.38
0.027 +		0.68	90	0.41	4.20		5.77
0.010 +		0.24	75	0.54	3.00		5.00
0.003 +		0.08	50	0.70	2.12		4.30
0.041 +	1.02 +	1.02	95	0.26	7.18	491.87	8.65
0.017 +	0.43 +	0.43	90	0.26	6.62	283.46	9.10
0.005 +	0.12 +	0.12	75	0.28	5.88	126.10	9.80
0.001 +	0.03 +	0.03	50	0.30	5.22	56.02	10.55

[a] All parameters given were calculated by using the dose-effect analysis computer program (5). CI values were calculated by using the mutually exclusive assumption as in references 7 and 40. The dose reduction index (DRI) (6) is the concentration of any of the MAbs alone required to effect a given level of neutralization divided by the concentration of that MAb in the combination required to effect that level of neutralization.

[b] MAbs 0.5β and 1125H were used at a 1:1 ratio when paired with each other and when combined with C108G in the three-MAb combination. Both 0.5β and 1125H were used at a 25-fold excess over C108G when each was paired with C108G and in the three-MAb combination.

activity against primary and/or monocytotropic isolates of HIV-1 (11). Encouragingly, the results shown here indicate that these anti-CD4 bs MAbs are unlikely to interfere with the neutralizing activities of anti-V2 and anti-V3 MAbs; rather, they appear to synergize with the latter even against some monocytotropic isolates. Despite the variability of both the V2 and V3 domains, there are relatively conserved epitopes within each (13, 16, 17) which may be targeted by vaccines and/or MAbs. Thus, elicitation of anti-V2 and anti-V3 neutralizing antibodies by vaccines and/or passive administration of cocktails of such MAbs to prevent and/or treat HIV-1 infection may be beneficial.

Theoretically, synergistic neutralization of HIV-1 by anti-V2 and anti-V3 and/or anti-CD4 bs antibodies could be achieved in vivo provided that the synergizing antibodies are present in appropriate ratios and that competing, poorly neutralizing or nonsynergizing antibodies do not interfere. Poorly neutralizing antibodies against a given epitope cluster, such as the V3 domain, are often those of lower affinity for virus (14, 26) and generally would not be expected to compete effectively with higher-affinity, more potent neutralizing antibodies. However, in vitro studies with MAbs suggest that those of certain epitope specificities and/or isotypes may be of high affinity for a given neutralization epitope cluster on virions and be able to compete with synergistically neutralizing antibodies, yet themselves be unable to participate in synergistic neutralization of a given virus (33, 38). Nevertheless, it has been demonstrated that anti-V3 neutralizing antibodies from sera of gp160 vaccinees could synergize with an anti-CD4 bs MAb in vitro (25). Further such studies as well as in vivo studies will be required to determine the feasibility of achieving significant synergism clinically between MAbs and antibodies against different neutralization epitope clusters of HIV.

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