

# Synergistic Neutralization of Simian-Human Immunodeficiency Virus SHIV-vpu<sup>+</sup> by Triple and Quadruple Combinations of Human Monoclonal Antibodies and High-Titer Anti-Human Immunodeficiency Virus Type 1 Immunoglobulins

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**We have tested triple and quadruple combinations of human monoclonal antibodies (MAbs), which are directed against various epitopes on human immunodeficiency virus type 1 (HIV-1) envelope glycoproteins, and a high-titer anti-HIV-1 human immunoglobulin (HIVIG) preparation for their abilities to neutralize a chimeric simian-human immunodeficiency virus (SHIV-vpu<sup>+</sup>). This virus encodes the HIV-1 strain IIB *env*, *tat*, *rev*, and *vpu* genes. The quantitative nature of the Chou-Talalay method (Adv. Enzyme Regul. 22:27–55, 1984) allows ranking of various combinations under identical experimental conditions. Of all triple combinations tested, the most potent neutralization was seen with MAbs 694/98D plus 2F5 plus 2G12 (directed against domains on V3, gp41, and gp120, respectively) as measured by the total MAb concentration required to reach 90% neutralization (90% effective concentration [EC<sub>90</sub>], 2.0 µg/ml). All triple combinations involving MAbs and/or HIVIG that were tested yielded synergy with combination index values of <1; the dose reduction indices (DRIs) ranged from 3.1 to 26.2 at 90% neutralization. When four MAbs (the previous three plus MAb F105, directed against the CD4 binding site) were combined, higher neutralization potency (EC<sub>90</sub>, 1.8 µg/ml) and a higher degree of synergy compared to any triple combination were seen. The mean DRIs of the quadruple combination were approximately twice that of the most synergistic triple combination. We conclude that human MAbs targeting different HIV-1 envelope glycoprotein epitopes exhibit strong synergy when used in combination, a fact that could be exploited clinically for passive immunoprophylaxis against HIV-1.**

Infection with the human immunodeficiency virus type 1 (HIV-1) will lead to AIDS in most cases if left untreated. During HIV-1 infection, neutralizing antibody responses that are directed against diverse epitopes on the HIV-1 envelope glycoprotein molecules gp120 and gp41 develop. In the initial stages of infection, the antibodies generated are mainly targeted against the linear neutralizing determinants in the third variable loop (V3) of gp120 (42). An early study showed that these antibodies neutralized a limited number of HIV-1 strains only (31), but further reports indicated that some anti-V3 antibodies reacted with less variable regions of V3 and exhibited a broader spectrum of HIV-1 neutralization (20, 23, 36). As HIV-1 infection progresses, antibodies directed against the CD4 binding site (CD4bd) and other complex epitopes develop that recognize discontinuous regions of gp120. These antibodies can neutralize diverse HIV-1 isolates (22, 25, 38, 44). Sera containing high-titer immunoglobulins to HIV type 2 (HIV-2) or simian immunodeficiency virus (SIV) have been

used successfully for passive protection of monkeys against challenge by homologous viruses (39).

Extensive work has been performed to develop human monoclonal antibodies (MAbs) directed against divergent HIV-1 envelope antigens. Some human MAbs potentially neutralized clinical HIV-1 isolates (4, 12, 20, 32, 35, 48). Combinations of human MAbs with different epitope specificities have shown additive or synergistic HIV-1 neutralization in vitro (2, 27, 45, 47, 50).

Animal models serve an important role in studying HIV pathogenesis and prophylaxis. In terms of clinical signs and laboratory findings, SIV infection of macaques mimics the natural course of HIV-1 infection in humans and thus is considered to be the best animal model (16). Owing to differences in envelope antigens between HIV-1 and SIV, human MAbs to HIV-1 cannot be studied in the SIV-macaque system. To overcome this barrier, SIV-HIV-1 chimeric viruses (SHIVs) were constructed that harbor HIV-1 *env*, *tat*, and *rev* genes in an SIV backbone. SHIVs replicate in macaque peripheral blood mononuclear cells (PBMC) (30, 40), infect monkeys, and, for some SHIV variants, cause lymphopenia or AIDS in infected animals (14, 24, 41).

In our previous report (29), we studied a panel of human MAbs and high-titer human anti-HIV-1 immunoglobulins

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(HIVIGs) for their abilities to neutralize SHIV-vpu<sup>+</sup>. The genome of this virus contains the *tat*, *rev*, *vpu*, and *env* genes of HIV-1 strain IIB; the remainder of the genome is derived from the SIV<sub>mac239</sub> backbone. SHIV-vpu<sup>+</sup> grows well in human T-cell lines (CEMx174 and MT-2) and in macaque PBMC (29, 30). Thus, it can serve as an ideal candidate in the macaque model to study passive immunoprophylaxis both in vitro and in vivo. We have shown that several human MABs neutralized SHIV-vpu<sup>+</sup> and that combinations of two effective MABs or MAB-HIVIG with different epitope specificities could act synergistically on the virus (29). Here, we report the interactions of human MABs or HIVIG when used in triple and quadruple combinations against SHIV-vpu<sup>+</sup>. The most potent virus neutralization and the highest degree of synergy were seen with a quadruple combination of human MABs.

## MATERIALS AND METHODS

**Human MABs and HIVIG.** In this study, we tested the following human MABs: F105, anti-CD4bd (37); 694/98D, anti-V3 domain (20); 2F5, anti-gp41 (35); and 2G12, directed against a complex gp120 epitope (49). All MABs are of the immunoglobulin G1 (IgG1) subclass, including 2F5 which had been engineered to contain the constant region of IgG1 instead of that of IgG3. HIVIG2, produced by Abbott Laboratories (Abbott Park, Chicago, Ill.) was obtained from the National Institute of Allergy and Infectious Diseases. A human IgG MAB, 860-30D, with irrelevant specificity (860-30D is directed against human cytomegalovirus and shows no cross-reactivity to HIV-1 or SIV) was used as a negative, isotype-specific control as single agent. No neutralization of SHIV-vpu<sup>+</sup> was seen (not shown).

**Preparation of SHIV-vpu<sup>+</sup> for neutralization.** An SHIV-vpu<sup>+</sup> stock was prepared in macaque PBMC (New England Regional Primate Research Center, Southboro, Mass.) as described elsewhere (29). The virus titer was 8,185 50% tissue culture infectious doses/ml.

**Virus neutralization assay.** We used an MT-2 cell viability assay (33) to measure the antibodies' capacities to neutralize SHIV-vpu<sup>+</sup> as described elsewhere (29). Briefly, antibodies at various dilutions (ranging from 8 to 16 μg/ml at the highest concentration), and combinations in triplicate wells were incubated with SHIV-vpu<sup>+</sup> at 37°C for 45 min. MT-2 cells were then added to the mixture. After incubation at 37°C with 5% CO<sub>2</sub> for 7 days, the degree of neutral red absorption, an indication of cell viability, was measured spectrophotometrically. The mean of two or three independent experiments was used as the final result.

The neutralization profiles of some MABs in PBMC from specific-pathogen-free (SPF) rhesus macaques (bred at the Yerkes Regional Primate Research Center, Atlanta, Ga.) were also tested. Reverse transcriptase activity (19) in cell culture supernatants was measured to define the percent neutralization.

**Determination of synergy and DRI.** The complex interactions of MABs and HIVIGs in triple and quadruple combinations were analyzed by computer software in a stepwise fashion, beginning with single agent dose-response curves, followed by dose-response curves involving combinations of two antibodies. Finally, the triple combination was calculated as a combination of antibody no. 1 in a two-component combination with antibody no. 2 plus antibody no. 3. A further step according to the same principle was used to calculate the quadruple combination of MABs. The analytical method of Chou-Talalay (7, 8) yields two parameters that describe the interactions among antibodies in a given combination: the combination index (CI) and the dose reduction index (DRI). A CI of <1 indicates synergism, a CI of 1 or close to 1 indicates additive effects, and a CI of >1 indicates antagonism.

DRI measures by what factor the dose of each drug in a combination may be reduced at a given effect level compared with the dose when each drug is used alone (10, 11). DRI may be influenced by the combination ratio and the number of drugs. Toxicity toward the host may be avoided or reduced when the dose is reduced.

The advantage of this method is that it takes into account not only the potency (median effect dose values [ $D_m$ ] or antibody concentration at 50% neutralization [ $EC_{50}$ ]), but also the shape (sigmoidicity) of the dose effect curve, based on the median effect equation of Chou (10):

$$f_a/f_u = (D/D_m)^m \quad (1)$$

Rearrangement of equation 1 gives

$$D = D_m [f_a/(1 - f_a)]^{1/m} \quad (2)$$

The logarithmic form of equation 1 gives

$$\log [f_a/f_u] = m \log(D) - m \log(D_m) \quad (3)$$

where  $D$  is dose (concentration of antibody), and  $f_a$  and  $f_u$  are the fractions of SHIV-vpu<sup>+</sup> affected (neutralized) and unaffected, respectively. Equation 2 al-

lows the calculation of  $D$  for  $x\%$  neutralization ( $D_x$ ) when the  $D_m$  and  $m$  values are determined from the median effect plot (10).

A plot of  $x = \log(D)$  versus  $y = \log [f_a/(1 - f_a)]$  (i.e., the median effect plot) yields the  $D_m$  values as  $x$  intercept antilog and the  $m$  value as the slope. Computer software developed by Chou (9) and Chou and Hayball (11), based on the median effect equation and the classic isobologram of Chou-Talalay (8), was used for automated analysis. Thus

$$CI = \sum_{n=1}^{2/3/4} \frac{(D)_n}{(D_x)_n} = \sum_{n=1}^{2/3/4} \frac{1}{(DRI)_n} \quad (4)$$

and the specified equation 2 gives

$$(D_x)_n = (D_m)_n [f_a/(1 - f_a)]^{1/m_n} \quad (5)$$

The correlation coefficient ( $r$ ) was also obtained from the dose effect analysis for the antibody combinations by using Calcsyn (11). Based on the experimental data,  $r$  values close to 1 indicate the conformity of the assays.

## RESULTS

**SHIV-vpu<sup>+</sup> neutralization in MT-2 cells by triple combinations of MABs.** We wished to extend the scope of our previous studies involving combinations of two antibodies (29) to test if triple or quadruple combinations of these MABs and HIVIG could further potentiate virus neutralization and increase synergy. Four human MABs with different epitope specificities were used in this experiment. The results of individual triple combinations are shown in Table 1. All four combinations showed significant synergy as judged by the low CI and high DRI values. The most potent neutralization was seen with the combination 694/98D plus 2F5 plus 2G12 (Table 1). At 90% neutralization, the total amount of MABs required was only 2.0 μg/ml and the CI reached 0.4. The combination of F105 plus 694/98D plus 2F5 reached the highest degree of synergy, as evidenced by a CI<sub>90</sub> of 0.3. These two combinations also yielded remarkable DRIs. The average DRI<sub>90</sub>s were 13.1 and 14.8, respectively (ranging from 4.6 to 26.2 [Table 1]). The other triple MAB combinations, namely, F105 plus 694/98D plus 2G12 and F105 plus 2F5 plus 2G12, exhibited similar synergy with lower potency. At 90% neutralization, the CIs were 0.4 and 0.5 and the mean DRIs were 8.9 and 14.8, respectively (Table 1).

**SHIV neutralization in MT-2 cells by a triple combination of MABs and HIVIG2.** The combination of F105 plus 694/98D plus HIVIG2 resulted in synergy (Table 1). HIVIG2, as a single agent, was significantly less potent than any neutralizing MAB tested. Consequently, a higher dose of this polyclonal preparation was needed in the combination, and, as a result, the total amount of antibodies required to produce significant neutralization was significantly higher compared to other triple MAB combinations. In this combination, 67 μg of antibodies per ml was needed to yield 90% neutralization, whereas in other MAB combinations, a mean total MAB amount of only 2.5 μg/ml was needed to yield the same degree of neutralization.

**Neutralization of SHIV-vpu<sup>+</sup> in macaque PBMC.** Since we intend to use the MAB combination regimens in vivo in macaques, we tested one MAB combination (F105 plus 2F5 plus 2G12) in PBMC of an SPF rhesus macaque. The results are shown in Table 2. Low CIs were observed, as shown previously for MT-2 cells. At 90% viral neutralization, the mean DRI was 10 (ranging from 4.5 to 20.3). The results obtained for macaque PBMC are consistent with those obtained for MT-2 cells (Table 1).

**SHIV neutralization in MT-2 cells by a quadruple combination of MABs.** Since any of the four MABs tested in various triple combinations acted synergistically, we postulated that a combination involving all four MABs would yield even higher

TABLE 1. Synergy of double and triple combinations of human IgG MAbs 694/98D, 2F5, 2G12, F105, and HIVIG2 for SHIV-vpu<sup>+</sup> neutralization in MT-2 cells

Single antibody (specificity) or antibody combination (ratio)	Concn (µg/ml) <sup>a</sup> for neutralization at:		CI <sub>90</sub> <sup>b</sup>	DRI <sup>c</sup> at EC <sub>90</sub> <sup>d</sup>			Mean DRI <sub>90</sub>
	50%	90%		MAb A	MAb B	MAb C	
<b>Single agents</b>							
694/98D (anti-V3)	1.7–1.9	NA <sup>e</sup>					
2F5 (anti-gp41)	0.5–0.6	3.5–3.9					
2G12 (anti-gp120)	0.4–0.5	1.6–3.2					
F105 (anti-CD4bd)	1.3–1.5	NA					
HIVIG2 (multiple)	33.6	NA					
<b>Combinations of two MAbs</b>							
694/98D + 2F5 (2:1)	0.5–0.7	2.3–3.0	0.3	12.3–14.3	4.0–4.7		
694/98D + 2G12 (2:1)	0.7	2.9–3.2	0.5–0.7	12.3	1.5–2.1		
2F5 + 2G12 (1:1)	0.3–0.4	1.7–2.1	0.7	2.6–3.9	2.1–2.7		
F105 + 2F5 (2:1)	0.6–0.8	3.1–3.8	0.4–0.6	9.1–11.4	2.1–3.8		
F105 + 2G12 (2:1)	0.6–0.7	2.9–3.2	0.6–0.7	8.6–9.4	1.7–2.2		
F105 + 694/98D (1:1)	1.0–1.3	6.4–8.2	0.3–0.5	4.2–5.9	3.6–8.1		
<b>Combination of MAbs and HIVIG2</b>							
F105 + HIVIG2 (1:25)	12.1	140.1	0.7	3.6	3.1		
694/98D + HIVIG2 (1:25)	13.4	84.1	0.4	5.1	5.2		
F105 + 694/98D + HIVIG2 (1:1:25)	8.2	67.0	0.4	7.8	6.7	6.7	7.1
<b>Combinations of three MAbs</b>							
694/98D + 2F5 + 2G12 (2:1:1)	0.4	2.0	0.4	26.2	8.6	4.6	13.1
F105 + 2F5 + 2G12 (2:1:1)	0.5	2.3	0.5	18.4	4.2	4.2	8.9
F105 + 694/98D + 2F5 (2:2:1)	0.7	3.0	0.3	19.1	18.9	6.3	14.8
F105 + 694/98D + 2G12 (2:2:1)	0.7	2.8	0.4	15.9	25.4	3.1	14.8

<sup>a</sup> The dose for neutralization by combination of two or three antibodies was the sum of that for each antibody used in the combination regimen.

<sup>b</sup> Synergy is indicated when the CI is <1.0. CIs were calculated based on the classical isobol equation (8, 10).

<sup>c</sup> DRIs were measured by comparing the doses required to reach a given degree of virus neutralization (i.e., 90%) when the antibody was used alone and in combination with other antibodies.

<sup>d</sup> Antibodies A, B, and C indicate the antibodies in first, second, or third position, respectively.

<sup>e</sup> NA, not achieved at the highest concentration tested.

synergy. The results of the F105 plus 694/98D plus 2F5 plus 2G12 combination are shown in Table 3. This quadruple combination yielded CIs even lower than those obtained from the triple combinations examined simultaneously. The CI<sub>90</sub> reached 0.2, and the mean DRI was 27.6 (ranging from 16 to 39.9), which is considerably better than the values obtained

from any of the triple combinations tested; the average DRI value of all of the MAbs in the four triple combinations tested was 11.7 in this assay (Table 3).

The mean *r* value for all of the triple and quadruple combinations was 0.983, which confirmed the consistency and reproducibility of the combination tests.

TABLE 2. Synergy of human IgG MAbs F105, 2F5, and 2G12 for SHIV-vpu<sup>+</sup> neutralization<sup>a</sup> in macaque PBMC

Single antibody (specificity) or combination (ratio)	Concn (µg/ml) for neutralization at:		CL <sub>90</sub> <sup>b</sup>	DRI at EC <sub>90</sub> <sup>c</sup>		
	50%	90%		MAb A	MAb B	MAb C
F105 (anti-CD4bd)	1.9	NA <sup>d</sup>				
2F5 (anti-gp41)	0.6	2.9				
2G12 (anti-gp120)	0.5	2.5				
F105 + 2F5 (2:1)	1.0	4.3	0.6	7.7	2.0	
F105 + 2G12 (2:1)	0.6	3.1	0.5	10.5	2.3	
2F5 + 2G12 (1:1)	0.4	2.0	0.8	2.7	2.3	
F105 + 2F5 + 2G12 (2:1:1)	0.4	2.1	0.5	20.3	5.3	4.5

<sup>a</sup> The dose for neutralization by combination of two or three antibodies was the sum of that for each antibody used in the combination regimen.

<sup>b</sup> Synergy is indicated when the CI is <1.0. CIs were calculated based on the classical isobol equation (8, 10).

<sup>c</sup> DRIs were measured by comparing the doses required to reach a given degree of virus neutralization (i.e., 90%) when the antibody was used alone and in combination with other antibodies. Antibodies A, B, and C indicate the antibodies in first, second, and third positions, respectively.

<sup>d</sup> NA, not achieved at the highest concentration tested.

TABLE 3. Synergy of human IgG MAbs F105, 694/98D, 2F5, and 2G12 for SHIV-vpu<sup>+</sup> neutralization<sup>a</sup> in MT-2 cells

Single MAb (specificity) or combination (ratio)	Concn ( $\mu\text{g/ml}$ ) for neutralization at:		CI <sub>90</sub> <sup>b</sup>	DRI at EC <sub>90</sub> <sup>c</sup>			
	50%	90%		MAb A	MAb B	MAb C	MAb D
F105 (anti-CD4bd)	1.3	NA <sup>d</sup>					
694/98D (anti-V3)	1.8	NA					
2F5 (anti-gp41)	0.6	5.1					
2G12 (anti-gp120)	0.5	4.9					
F105 + 694/98D (1:1)	1.4	8.9	0.4	5.2	5.5		
F105 + 2F5 (2:1)	0.8	5.3	0.5	6.5	2.9		
F105 + 2G12 (2:1)	0.8	4.7	0.5	7.4	3.1		
694/98D + 2F5 (2:1)	0.7	3.7	0.4	9.9	4.1		
694/98D + 2G12 (2:1)	0.8	4.0	0.4	9.1	3.7		
2F5 + 2G12 (1:1)	0.4	1.8	0.4	5.6	5.5		
F105 + 694/98D + 2F5 (2:2:1)	0.8	3.6	0.3	15.4	16.3	6.7	
F105 + 694/98D + 2G12 (2:2:1)	0.9	3.5	0.3	16.7	17.6	7.1	
F105 + 2F5 + 2G12 (2:1:1)	0.6	2.8	0.4	16.1	7.0	6.8	
694/98D + 2F5 + 2G12 (2:1:1)	0.5	2.7	0.3	17.2	7.1	6.9	
F105 + 694/98D + 2F5 + 2G12 (2:2:1:1)	0.5	1.8	0.2	37.9	39.9	16.5	16

<sup>a</sup> The dose for neutralization by combination of two, three, or four antibodies was that for the sum of each antibody used in the combination regimen.

<sup>b</sup> Synergy is indicated when the CI is <1.0. CIs were calculated based on the classical isobol equation (8, 10).

<sup>c</sup> DRIs were measured by comparing the doses required to reach a given degree of virus neutralization (i.e., 90%) when the antibody was used alone and in combination with other antibodies. Antibodies A, B, C, and D indicate the antibodies in first, second, third, and fourth positions, respectively.

<sup>d</sup> NA, not achieved at the highest concentration tested.

## DISCUSSION

In the present study, we have shown that combinations of three or four effective human MAbs or HIVIG acted synergistically and completely neutralized SHIV-vpu<sup>+</sup>, even in rhesus macaque PBMC. For the first time, the quantitative interaction of MAbs in a quadruple combination has been calculated; the latter was the most potent among all regimens tested and revealed the highest degree of synergy.

The MAbs chosen for this experiment have different characteristics. MAb F105 is directed against the CD4bd (37) and neutralized laboratory and primary isolates of HIV-1 (38). The lack of native antibodies which shared the binding epitopes with F105 in the serum of HIV-infected patients correlated with disease progression (5). The anti-V3 MAb 694/98D not only neutralized several laboratory isolates of HIV-1 effectively (20, 21) but also activated complement (44). MAb 2G12 defines a distinctive but discontinuous neutralization epitope on HIV-1 gp120. It recognizes domains in the C2 and C3 regions near the base of the V3 loop and domains in the V4 loop and C4 region (49). In cross-competition experiments, 2G12 neither cross-blocked nor was cross-blocked by 45 other MAbs which recognized continuous or discontinuous gp120 epitopes, including the V2 and V3 loops; the C1, C4, and C5 regions; discontinuous epitopes overlapping the CD4bd; and CD4-induced epitopes (34). Moreover, some MAbs against V3, C4, and CD4bd-related epitopes modestly increased the binding of 2G12 to gp120 (34). Another MAb tested in this series of experiments was 2F5, which interacts with the amino acid sequence ELDKWA on the ectodomain of gp41 (35). Since this sequence is present in 72% of HIV-1 isolates of different clades, 2F5 is broadly reactive against various HIV-1 isolates (35). Both 2G12 and 2F5 have shown a potent ability to neutralize divergent clinical isolates of HIV-1 *in vitro* (12, 48). MAb 2F5 delayed the appearance of viremia and reduced virus load when given passively to chimpanzees before HIV-1 challenge (13).

Combinations of two human MAbs with different epitope specificities have shown additive or synergistic effects on laboratory-adapted as well as clinical isolates of HIV-1 (3, 27, 29, 45, 47, 50). Combining anti-CD4bd MAbs with anti-V3 MAbs synergistically neutralized HIV-1 (27, 45, 47). A recent study also reported synergy in the triple combination of anti-V2, anti-V3, and anti-CD4bd MAbs (50). These results all indicate that MAbs with different epitope specificities and at appropriate concentrations act synergistically on HIV-1, leading to more potent neutralization. The results of our study are in accordance with these findings. All triple MAb combinations reached significant synergy and DRIs. The quadruple MAb combination showed even better CIs and DRIs.

In comparison to the potency of the neutralizing human MAbs tested, the performance of HIVIG2 was somewhat disappointing in our assays, which could be explained by the relatively low abundance of highly neutralizing antibodies, the possible presence of infection-enhancing antibodies, or the presence of antibodies that interfere with neutralizing antibodies. Given the polyclonal nature of HIVIG2, we can only measure the overall neutralization capacity but not that of individual components contained in this mixture. Our strategy of combining human MAbs with known neutralization profiles is designed to avoid the problem of antibody interference and to potentiate virus neutralization.

The mechanism for the synergy in MAb-HIVIG combinations has not been fully elucidated. However, MAb binding studies have shown that conformational changes of the antigens during antigen-antibody interaction may make antigenic domains on the envelope glycoprotein more accessible to MAbs, thus facilitating MAb-antigen interaction (6, 47, 49, 51). We postulate that the antibody-virus interactions in our neutralization experiments also induced conformational changes in the envelope glycoprotein of SHIV-vpu<sup>+</sup>, which may explain the synergy observed.

Neutralizing human MAbs against HIV-1 have been studied

extensively, given their potential application for passive prevention of infection in neonates born to HIV-1-positive mothers and healthcare workers or researchers accidentally exposed to the virus. Some MAbs with potent neutralizing effects have been tested extensively *in vitro* (4, 12, 46, 48, 49) and could be candidates for passive immunoprophylaxis.

Passive immunoprophylaxis has been tested in several animal models, with conflicting outcomes. Some studies have yielded promising results, whereas others failed to show protection against virus challenge. Sera with high titers of antibodies against the V3 loop of HIV-2 or SIV protected monkeys against infection after challenge with HIV-2 or SIV<sub>sm</sub>, respectively (39). When an anti-HIV-1 V3 MAb was given to chimpanzees before or shortly after challenge with HIV-1, the animals were protected (15). Administration of serum from a cynomolgus monkey, which had been immunized with inactivated whole HIV-2 and resisted homologous virus challenge, protected some but not all monkeys against challenge with HIV-2 or SIV<sub>sm</sub> (1), and three of four monkeys given SIV<sub>sm</sub> antiserum remained uninfected after challenge with homologous cell-free virus (1). Lewis et al. (28) tested passive prophylaxis with inactivated plasma derived from SIV-infected rhesus macaques or from animals given a peptide vaccine; half of the recipients of the SIV-peptide vaccine plasma and 82% of the animals treated with plasma from infected monkeys did not seroconvert after SIV challenge. When the protected monkeys were challenged a second time, a third of them remained uninfected (28). Gardner et al. (17) reported the success of passive immunization with plasma from a monkey protected after vaccination with inactivated whole SIV<sub>mac</sub>. The recipient monkeys were either completely or partially protected against intravenous challenge with 10–50% animal infectious doses of homologous cell-free virus. In contrast to these studies, inactivated plasma or purified immunoglobulins derived from monkeys chronically infected with SIV<sub>mac</sub> not only failed to protect but may have even facilitated infection and accelerated disease progression (18). Passive administration of MAb 2F5, which is directed against an epitope on gp41, failed to protect recipient chimpanzees against challenge with primary HIV-1 strains (13). However, the peak viral RNA production was delayed in one recipient and remained significantly lower in a second one compared to controls, indicating that passive immunoprophylaxis may have altered the course of viremia in these chimpanzees (13). Failure of passive immunoprophylaxis with either neutralizing MAbs or sera against SIV challenge was reported in a number of studies (26, 43). Our strategy to use combinations of effective neutralizing MAbs may eliminate the unpredictable influence of infection-enhancing antibodies of polyclonal sera from immunized or infected individuals and result in synergistic virus neutralization.

Our data and those of others raise the hope that maternal-infant transmission of HIV-1 could be blocked with synergistic combinations of effective human MAbs. Indeed, all antibodies tested in our series of experiments are of the IgG isotype and are expected to cross the placenta. Theoretically, the neutralizing MAb levels could be achieved in the fetus by passive immune therapy of the pregnant woman. We plan to test this hypothesis in our SHIV-rhesus macaque model of neonatal mucosal challenge, in which we have not only achieved reproducible oral infection but also observed disease (unpublished data).

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