Lack of Enhancing Effect of Human Anti-Human Immunodeficiency Virus Type 1 (HIV-1) Antibody on HIV-1 Infection of Human Blood Monocytes and Peritoneal Macrophages

PHILLIP P. SHADDUCK,¹ J. BRICE WEINBERG,^{2,3} ARTHUR F. HANEY,³ JOHN A. BARTLETT,²
ALPHONSE J. LANGLOIS,¹ DANI P. BOLOGNESI,^{1,4} AND THOMAS J. MATTHEWS^{1*}

Center for AIDS Research, Department of Surgery,¹ Department of Medicine,² Department of Obstetrics and Gynecology,³ and Department of Microbiology and Immunology,⁴ Duke University and VA Medical Centers, Durham, North Carolina 27710

Received 31 December 1990/Accepted 18 April 1991

The influence of human anti-human immunodeficiency virus type 1 (HIV-1) antibody on HIV-1 infection of freshly isolated normal human peritoneal macrophages and blood monocytes was examined. Each of 14 HIV antibody-positive human serum samples was found to block the infection of four virus isolates (human T-cell lymphotropic virus type III_{Ba-L} [HTLV-III_{Ba-L}], HTLV-III_B, D.U. 6587-7, and D.U. 7887-8) at serum dilutions ranging from 10⁻¹ to 10⁻². Three of these isolates (HTLV-III_{Ba-L}, D.U. 6587-7, and D.U. 7887-8) infected cultures of monocytes and macrophages rapidly and produced high levels of virus reverse transcriptase and p24 antigen. A fourth virus isolate (HTLV-III_B) infected the monocytes and macrophages more slowly and produced low levels of viral protein. More dilute HIV antibody-positive sera had no significant effect on the overall level or rate of virus infection or expression. Complement did not appear to influence the course of infection by any combination of antisera or virus examined. Successful HIV-1 infection of the peritoneal macrophages and blood monocytes under the conditions tested showed strict dependence on CD4 since a recombinant CD4 polypeptide and an anti-CD4 monoclonal antibody effectively blocked the process.

The role of the humoral immune response in human immunodeficiency virus type 1 (HIV-1) infections is not well understood. Although it has been demonstrated that antibodies can neutralize HIV-1 infection both in vitro (25, 29, 39, 54) and in vivo (8, 35), there is also in vitro evidence that antibodies may facilitate HIV-1 infection under some conditions (14, 16–18, 27, 34, 40–43, 45, 50, 56). The enhancing effect has been shown to be operative in both human cell lines and cultured blood mononuclear cells. At least two mechanisms appear to be involved. One is mediated by antibody and Fc receptors (FcR) (17, 50), and the other is mediated by antibody, complement, and type 2 complement receptors (CR2) (41). The purpose of our study was to determine the effects of anti-HIV-1 antibody on the HIV-1 infection of freshly isolated normal (primary) human peritoneal macrophages and blood monocytes.

MATERIALS AND METHODS

Sera. Peripheral venous blood was collected in sterile, additive-free Vacutainer tubes (Becton Dickinson, Mountain View, Calif.) from 14 HIV-1-seropositive donors and 3 seronegative normal controls. Seropositive donors spanned Walter Reed (WR) stages 2 to 6 and possessed absolute T4 counts of 12 to 840 cells per mm³ (WR 2 = patients 5, 8, 10, 11, 13, and 14; WR 3 = patients 4, 6, and 7; WR 4 = patient 3; WR 5 = patient 1; WR 6 = patients 2, 9, and 12) (37). Blood was allowed to clot at room temperature. Serum was removed, passed through 0.45- μ m-pore-size filters (Millipore, Continental Water Systems, Bedford, Mass.), and tested fresh on the day of collection. Additionally, paired fresh and heat-inactivated (56°C for 30 min) aliquots of four serum samples were tested in parallel, and three serum

samples were further tested in the presence and absence of exogenous human complement (Sigma Chemical Co., St. Louis, Mo.).

Cells. Blood monocytes were isolated from heparinized peripheral venous blood from HIV-seronegative healthy donors by sequential Ficoll-Hypaque (Sigma) and Percoll (Pharmacia, Uppsala, Sweden) gradient sedimentation as described previously (52). Human peritoneal macrophages were obtained by harvesting the free peritoneal fluids of seronegative infertile women undergoing diagnostic laparoscopy (52), and cells were isolated by Ficoll-Hypaque sedimentation. Blood monocytes and peritoneal macrophages were suspended in serum-free Dulbecco's modified Eagle's medium (GIBCO Laboratories, Grand Island, N.Y.) at a density of either 3 $\times$ 10⁶ cells per ml (monocytes) or 2 $\times$ 10⁶ cells per ml (macrophages), and 100 μ l per well was plated in 96-well tissue-culture-treated polystyrene clusters (Costar, Cambridge, Mass.). After a 1-h incubation at 37°C to allow monocyte/macrophage adherence, cells were washed with sterile phosphate-buffered saline (PBS) and thereafter maintained at 37°C in 200 μ l of Dulbecco's modified Eagle's medium containing 10 to 15% unheated human serum, 100 U of penicillin (GIBCO) per ml, and 100 μ g of streptomycin (GIBCO) per ml. Peritoneal macrophages were infected 2 to 4 days after isolation (average, 3 days), and blood monocytes were infected 1 to 6 days after isolation (average, 4 days). Monocyte/macrophage purity was assessed by Wright's staining, nonspecific esterase staining, and phagocytosis of *Candida albicans* (52, 53). Cell monolayers were also stained for CD11b (OKM1; Ortho Diagnostics, Raritan, N.J.) by previously described techniques (53). For p24 antigen staining, monocyte monolayers were established from 10⁶ monocytes per chamber on eight-chambered Lab-Tek slides with chambers of 1-cm² surface area (Lab-Tek, Naperville, Ill.). Cells were exposed to HIV-1_{Ba-L}, cultured for 14 days, fixed

* Corresponding author.

with acetone, and stained with murine anti-p24 monoclonal antibody and fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin G by standard indirect immunofluorescence techniques (53).

Virus. The human T-cell lymphotropic virus type III_{Ba-L} (HTLV-III_{Ba-L}) (Ba-L) and HTLV-III_B (IIIB) isolates have been described previously (10). The D.U. 6587-7 and D.U. 7887-8 strains were isolated by A. J. Langlois and K. J. Weinhold (Duke University) from peripheral blood lymphocytes. These two isolates were expanded by two passages through the human cell line CEM. The D.U. 6587-7 pool contained 10^3 100% tissue culture infective doses (TCID₅₀/ml) on monocytes, 43,000 cpm of reverse transcriptase (RT) activity per ml, and 6 ng of p24 antigen per ml. The D.U. 7887-8 pool contained 10^5 TCID₅₀/ml on monocytes, 919,000 cpm of RT activity per ml, and 75 ng of p24 antigen per ml. The Ba-L virus stock was prepared from chronically infected CEM cells and contained 2×10^4 TCID₅₀/ml on monocytes, 116,000 cpm of RT activity per ml, and 27 ng of p24 antigen per ml. The IIIB stock was prepared from chronically infected H9 cells and contained 2×10^3 TCID₅₀/ml on CEM, 379,000 cpm of RT activity per ml, and 40 ng of p24 antigen per ml. All virus pools were prepared by passing cell-free culture supernatants through 0.45- μ m-pore-size filters (Millipore) and were stored in aliquots at -70°C until use.

Infectivity assay. Serial dilutions of test and control sera were prepared in Dulbecco's modified Eagle's medium and added in duplicate to microtiter wells such that final serum dilutions were 10^{-1} to 10^{-8} . Exogenous human complement was reconstituted according to the manufacturer's instructions and added with sera such that the final complement dilution was 1:80. A soluble recombinant CD4 polypeptide (sT4; Smith Kline & French Laboratories, King of Prussia, Pa.) (6) and anti-CD4 monoclonal antibody 19Thy5D7 (a generous gift from Ellis Reinherz) (47) were tested in parallel with sera. Approximately 150 TCID₅₀ of virus were then added per well. As indicated in the text, virus inocula in certain experiments were 4 and 20 TCID₅₀ per well. Medium was completely removed from all wells 1 to 5 days after infection, cells were washed once with PBS or medium, and fresh culture medium was added. On indicated days, medium was removed from the wells, treated with Triton X-100 (1% final), and stored at -20°C until tested for p24 antigen content or RT activity.

p24 assay. p24 antigen determinations were done with commercial kits according to the manufacturers' instructions (Du Pont Co., Wilmington, Del., and Organon Teknika-Cappel, Durham, N.C.).

RT assay. Cell-free culture supernatants were assayed for RT activity by a modified micro-RT assay (13, 55). Briefly, 10 μ l of Triton-treated culture supernatant was added to 50 μ l of RT reaction cocktail containing 10 μ Ci of [³²P]dTTP (Amersham) per ml, 5 μ g of poly(A) (Pharmacia) per ml, 0.03 A₂₆₀ units of oligo(dT) (Pharmacia) per ml, 50 mM Tris hydrochloride buffer (pH 7.8) (Boehringer Mannheim Biochemicals, Indianapolis, Ind.), 2 mM dithiothreitol (Calbiochem, La Jolla, Calif.), 75 mM KCl, 5 mM MgCl₂, and 0.05% Nonidet P-40 detergent in distilled water. After a 90-min incubation at 37°C , 40 μ l of each sample was spotted onto DEAE paper (DE-81; Whatman International, Maidstone, England), and unincorporated [³²P]dTTP was washed from the paper with 0.3 M NaCl-0.03 M Na citrate. Papers were then cut and placed in distilled water, and their radioactivities were counted in a liquid scintillation counter (Packard Instrument Co., Sterling, Va.).

RESULTS

Characteristics of human peritoneal macrophages and blood monocytes. Monocyte and macrophage monolayers were >95% pure as determined by light microscopic appearance (Wright's stain), nonspecific esterase staining, and phagocytosis of *C. albicans*. Monocytes were greater than 90% CD11b-positive by immunofluorescent staining. Monocyte morphology by light microscopy was consistent with previous reports (21, 52, 53, 57). Briefly, monocytes were well spread in the initial hours and then less well spread (decreased cytoplasm) for 3 to 5 days. Subsequently, they became larger and more spread and remained viable for greater than 4 weeks. Compared with blood monocytes, peritoneal macrophages were larger at the time of isolation, spread more rapidly on the plastic (53), and remained well spread throughout the culture. Both blood monocytes and peritoneal macrophages were highly susceptible to HIV-1 infection, with 70 to 90% of cells staining positive for cytoplasmic HIV p24 antigen within 2 weeks of exposure to Ba-L (Fig. 1). With two HIV-1 isolates (Ba-L and D.U. 6587-7), HIV p24 antigen and RT appeared in culture supernatants 5 to 7 days after infection and peaked 10 to 14 days after infection (Fig. 2). The kinetics of infection were intermediate with a third HIV-1 isolate (D.U. 7887-8; data not shown) and slower with a fourth HIV-1 isolate (IIIB) (Fig. 2). HIV-induced cytopathicity and viral antigen production tended to occur slightly earlier in peritoneal macrophages than in blood monocytes, although kinetics varied depending on the cell donor.

Soluble CD4 and 19Thy5D7 blocked primary monocyte/macrophage infection. To determine whether HIV-1 infection of the primary blood monocytes and peritoneal macrophages required CD4, we exposed cells to HIV-1 (isolate Ba-L) in the presence and absence of a soluble recombinant CD4 polypeptide (sT4) and the anti-CD4 monoclonal antibody 19Thy5D7. These agents block binding of HIV-1 to CD4, HIV-1 infection of CD4-positive T-cell lines, and HIV-induced cell-cell fusion (6, 47). Infection of blood monocytes and peritoneal macrophages was inhibited 94 to 100% by sT4 at concentrations of ≥ 5 μ g/ml and by 19Thy5D7 at concentrations of ≥ 10 μ g/ml (Fig. 3). These findings suggest that a CD4-dependent mechanism of HIV-1 infection was operative in these primary cells.

HIV antibody-positive human sera neutralized and did not enhance monocyte infection. The effect of HIV antibody-positive human sera on the HIV-1 infection of primary human monocytes and macrophages was then studied. When tested on blood monocytes, each of 12 HIV antibody-positive serum samples neutralized HIV-1 infection at low serum dilutions (Fig. 4). HIV antibody-positive sera inhibited production of HIV p24 antigen and RT by $99\% \pm 1\%$ at serum dilutions of 10^{-1} to $10^{-1.6}$, and by $86\% \pm 13\%$ at a serum dilution of 10^{-2} . Results were similar with all four HIV-1 isolates examined (Ba-L, IIIB, D.U. 7887-8, and D.U. 6587-7). With all combinations of sera and virus isolates, subneutralizing concentrations of human sera had no significant effect on the rate or level of virus production (Fig. 4). Specifically, the ratio of HIV p24 antigen and RT produced by blood monocytes infected in the presence of HIV antibody-positive human sera at dilutions of 10^{-3} through 10^{-8} to the amount produced by parallel controls infected in the presence of equal concentrations of normal human sera averaged 0.78 ± 0.29 (range, 0.24 to 2.40). In three instances, the ratios of virus production in the presence of subneutralizing dilutions of HIV antibody-positive

FIG. 1. Light microscopic appearance and cytoplasmic p24 antigen staining of blood monocytes. Human blood monocytes were plated on Lab-Tek slides, infected with Ba-L, cultured for 14 days, and stained for cytoplasmic HIV p24 antigen as described in the text. Light (phase) microscopic appearance (A and B) and p24 antigen (immunofluorescent) staining (C and D) are shown for typical uninfected (A and C) and HIV-infected (B and D) cells.

sera were high (1.59, 1.67, and 2.40), as indicated, for example, in Fig. 4 by virus D.U. 7887-8, in which the 10^{-5} dilution of serum 1 give a greater p24 signal than control serum at that dilution. Each of these three specific virus-serum dilution combinations was retested three times. The results showed average corresponding ratios of virus production for each of the conditions as 0.89, 1.0, and 0.83, respectively. Thus, none of 12 HIV antibody-positive human serum samples tested on blood monocytes reproducibly

increased HIV p24 antigen or RT production over that of controls.

HIV antibody-positive sera have the same effect on tissue macrophages as on blood monocytes. To determine whether the influence of antibody on virus infection might depend on the state on monocyte/macrophage differentiation, we also tested 11 HIV antibody-positive serum samples for their

FIG. 2. Kinetics of blood monocyte infection by three HIV-1 isolates. Blood monocytes were isolated and infected with three HIV-1 isolates (Ba-L, D.U. 6587-7, and IIIB) as described in the text. Supernatants were sampled on the indicated days and assayed for RT activity. The rate and level of HIV antigen production by a fourth HIV-1 isolate (D.U. 7887-8) was intermediate between that of isolates D.U. 6587-7 and IIIB (data not shown).

FIG. 3. Effect of sT4 and 19Thy5D7 on peritoneal macrophage infection. Human peritoneal macrophages were isolated and infected with Ba-L in the presence of a soluble recombinant CD4 polypeptide (sT4) and an anti-CD4 monoclonal antibody (19Thy5D7) as described in the text. sT4 and 19Thy5D7 were added at the time of virus inoculation and were not replaced with medium changes on days 7, 10, and 14. p24 antigen concentrations are shown for supernatants harvested 10 days after exposure to virus.

FIG. 4. Effect of HIV antibody-positive human sera on the infection of blood monocytes by four HIV-1 isolates. Blood monocytes were isolated and infected with four HIV-1 isolates (Ba-L, IIIB, D.U. 6587-7, and D.U. 7887-8) as described in the text. HIV antibody-positive human sera were tested at the final dilutions indicated. Sera shown for isolate Ba-L were obtained from patients 12 (●), 13 (▲), and 14 (◆) and normal control donor 2 (■). Sera shown for isolate IIIB were obtained from patients 7 (●), 8 (▲), 9 (◆), and 10 (▲) and normal control donor 2 (■). Sera shown for isolate D.U. 7887-8 were obtained from patients 1 (●) and 3 (▲) and normal control donor 1 (■). Sera shown for isolate D.U. 6587-7 were obtained from patients 1 (●) and 3 (▲) and normal control donor 2 (■). Data are shown for supernatants harvested 7 days (Ba-L, D.U. 6587-7), 8 days (D.U. 7887-8), and 16 days (IIIB) after infection.

effect on the HIV-1 infection of freshly isolated normal human peritoneal macrophages. As with blood monocytes, the infection of peritoneal macrophages was blocked by low dilutions of HIV antibody-positive sera (Fig. 5). HIV p24 antigen and RT production were inhibited $97\% \pm 8\%$, $89\% \pm 18\%$, and $64\% \pm 29\%$ by HIV antibody-positive sera tested

FIG. 5. Effect of HIV antibody-positive human sera on the infection of peritoneal macrophages. Human peritoneal macrophages were isolated and infected with Ba-L as described in the text. Sera were obtained from patients 1 (●), 2 (▲), 3 (△), and 4 (○) and normal control donor 2 (■). Data are shown for supernatants harvested 9 days after infection. Data were similar with seven additional HIV antibody-positive human serum samples.

at final serum concentrations of 10^{-1} , $10^{-1.6}$, and 10^{-2} , respectively. Higher dilutions of sera did not definitively alter the course of infection. Specifically, the ratio of virus antigen produced in test wells containing HIV antibody-positive sera at concentrations of $\geq 10^{-3}$ compared to control wells containing normal human sera at the same concentrations averaged 0.98 ± 0.19 (range, 0.42 to 1.36).

Complement did not increase HIV-1 infection of monocytes or macrophages. Because HIV antibody-positive human sera have been shown to enhance HIV-1 infection of MT-2 cells when tested in the presence of complement (40-43, 45), we looked for a similar effect of complement on the Ba-L infection of primary blood monocytes and peritoneal macrophages. Paired fresh (complement-sufficient) and heat-inactivated (complement-depleted) aliquots of four HIV antibody-positive human serum samples were tested in parallel. No significant differences in viral antigen production were observed in the presence of complement (Fig. 6a). Three heat-inactivated (complement-depleted) serum samples were further tested in the presence and absence of an exogenous source of human complement, and again no differences were observed (Fig. 6b). The complement preparation utilized here was obtained from the same source and tested at the same concentration as in the MT-2 cell studies. Assays were repeated with two additional HIV-1 isolates (D.U. 6587-7 and D.U. 7887-8) and one additional antibody-positive serum sample, and results were similar to those shown.

HIV antibody-positive human sera did not alter kinetics of monocyte infection. Because HIV antibody-positive human sera have been shown to increase the rate of HIV-1 infection

FIG. 6. Effect of complement on the HIV-1 infection of human peritoneal macrophages and blood monocytes. (a) Human peritoneal macrophages were infected as described in the text with Ba-L in the presence of paired fresh (---) and heat-inactivated (—) aliquots of HIV antibody-positive human sera. Sera were obtained from patients 5 ($\square$) and 6 ($\circ$) and normal control donor 2 ($\blacksquare$). The p24 antigen contents of supernatants sampled 8 days after infection are shown. (b) Blood monocytes were infected as described in the text with Ba-L in the presence of complement-depleted (heat-inactivated) HIV antibody-positive human sera tested in the presence (---) and absence (—) of exogenous human complement serum (Sigma; 1:80 final dilution). Sera shown were obtained from patients 3 ($\square$) and 4 ($\circ$) and normal control donor 1 ($\blacksquare$). The p24 antigen contents of supernatants sampled 8 days after infection are shown.

in the human cell line U937 (50, 56), we looked for a similar kinetic effect of antibody on primary blood monocytes. Cell culture medium was completely changed 2 days after infection, and supernatants were then harvested every 1 to 2 days for 2 weeks. RT production in the presence and absence of antibody was assessed as a function of time. Even at the earliest time points, RT production was not significantly affected by subneutralizing concentrations of three immune human serum samples (Fig. 7).

Effect of HIV antibody-positive human sera did not depend on virus inoculum. Finally, because it has been reported that some human antibody preparations neutralize HIV-1 infection when tested with moderate virus inocula yet facilitate infection when tested with very low virus inocula (34), we examined the effect of two HIV antibody-positive human serum samples on the Ba-L infection of primary blood monocytes at limiting dilutions of virus (4 and 20 TCID₅₀s per well). Although at limiting dilutions of virus there was more variability in RT production among replicate wells (five per condition) than at higher virus inocula, RT production in

FIG. 7. Effect of HIV antibody-positive human sera on the early kinetics of blood monocyte infection by HIV-1. Blood monocytes were infected with isolate D.U. 6587-7 in the presence of HIV antibody-positive human serum (obtained from patient 3) at final serum dilutions of 10^{-1} through 10^{-5} . Virus production in the presence of no serum ($\blacksquare$) is shown for comparison, along with standard deviation bars (10 replicate wells). Similar results were obtained with two additional HIV antibody-positive sera (obtained from patients 1 and 12; data not shown).

the presence of HIV antibody-positive sera was not significantly higher than that in the presence of either control human sera or no human sera (Table 1; analysis of variance, P was not significant at 0.05).

DISCUSSION

In vitro, anti-HIV antibodies prevent HIV-1 infection under some conditions (25, 29, 39, 54) and facilitate infection under other conditions (14, 16–18, 27, 34, 40–43, 45, 50, 56). Although as yet undemonstrated (7–9, 12, 19, 26, 32, 35, 49), in vivo correlates to the latter activity would have a major impact on the development of HIV-1 vaccines and passive immunotherapies (1, 44). Most of what is known about the functional activity of anti-HIV antibodies has come from the study of human lymphocytic and monocytic cell lines. Less is known about the effect of antibody on the HIV-1 infection of freshly isolated normal human cells, particularly human

TABLE 1. Effect of HIV antibody-positive human sera on the infection of blood monocytes by a low inoculum of HIV-1^a

Serum	Dilution	RT activity (cpm/6 μ l)	
		Day 7	Day 10
Patient 8	10^{-2}	69 $\pm$ 66	324 $\pm$ 324
	10^{-5}	185 $\pm$ 129	689 $\pm$ 254
	10^{-6}	209 $\pm$ 84	779 $\pm$ 406
	10^{-7}	312 $\pm$ 272	1,343 $\pm$ 498
Patient 9	10^{-2}	19 $\pm$ 16	302 $\pm$ 311
	10^{-5}	114 $\pm$ 46	821 $\pm$ 241
	10^{-6}	258 $\pm$ 101	1,028 $\pm$ 573
	10^{-7}	359 $\pm$ 327	1,354 $\pm$ 826
Seronegative control	10^{-2}	136 $\pm$ 85	1,006 $\pm$ 451
	10^{-5}	242 $\pm$ 172	962 $\pm$ 254
	10^{-6}	364 $\pm$ 363	1,334 $\pm$ 634
	10^{-7}	389 $\pm$ 270	1,448 $\pm$ 339

^a Four TCID₅₀s of Ba-L per well. Relative RT production between HIV antibody-positive patient sera and seronegative control sera was similar with 20 TCID₅₀s of Ba-L per well.

blood monocytes and tissue macrophages. This study examined these latter cell types.

Serum samples from 14 HIV-infected humans, four HIV-1 isolates, and two primary human mononuclear phagocyte populations were studied. Because the stage of HIV-1 infection has been reported to affect both the titer and function of HIV-specific antibodies (16, 24, 30), serum samples were collected from both early and late stage patients. Also, since it has been suggested that the neutralizing-enhancing activity of antibody is isolate dependent (3, 16, 36), four biologically distinct HIV-1 isolates were examined, two prototypic isolates (Ba-L and IIB) and two field isolates (D.U. 6587-7 and D.U. 7887-8). Finally, to determine whether the stage of mononuclear phagocyte differentiation might influence the results, we studied both blood monocytes and tissue macrophages (peritoneal). In all assays, high concentrations of HIV antibody-positive sera blocked infection of both monocytes and macrophages. Subneutralizing concentrations of sera had no significant effect on either the overall level or the rate of infection. Finally, successful HIV-1 infection of primary macrophages under the conditions tested showed strict dependence on CD4 since a recombinant CD4 polypeptide and an anti-CD4 monoclonal antibody effectively blocked the process.

It has been shown that HIV-1 infection of the HTLV-I-transformed T-cell line MT-2 is enhanced by most HIV-positive human sera when appropriate serum dilutions are tested in the presence of human complement (41). This phenomenon involves CD4, HIV envelope-specific antibodies, complement component C3d, and the cellular complement receptor CR2, a molecule that is expressed at particularly high levels on MT-2 cells (41). Inasmuch as primary human monocytes and macrophages express little if any CR2 (4, 46, 48), it is not surprising that this complement-dependent effect was not observed in the experiments described in the present study. Whether a related phenomenon is operative in primary B lymphocytes, the major human cell population which expresses the CR2 receptor (4, 46, 48), or in cell populations which coexpress CD4 and CR2, such as follicular dendritic cells (11, 38), remains to be established.

Takeda et al. (50) have shown that HIV antibody-positive human sera also increase the rate of HIV-1 infection in the monocytic cell line U937, although by a different mechanism than the one operative in MT-2 cells. The effect on U937 cells appears to be mediated by CD4, low concentrations of immunoglobulin G (serum dilution equivalents of 10^{-3} to 10^{-6}), and high-affinity cellular receptors for immunoglobulin (FcR I). Similar results have been reported by Zeira et al. (56) and Matsuda et al. (27). However, in other studies with the U937 cell line, HIV-specific human antibodies were not found to increase infection, although they did mediate binding of envelope gp120 to these FcR-bearing cells (22). Additionally, a human Fc-murine Fab chimeric monoclonal antibody which binds to the principal neutralizing determinant of the IIB envelope has also been tested on U937 cells and found not to increase IIB infection (28). In the studies described here utilizing primary blood monocytes and peritoneal macrophages, cell populations which express both CD4 (5) and FcR I (51), we also were unable to demonstrate a kinetic, enhancing effect of antibody.

The studies with the MT-2 and U937 cell lines, as well as other recent reports (31), have raised a number of basic questions regarding the mechanism(s) of HIV-1 entry. In addition to the studies reported here, several groups have begun to explore these questions using primary human monocyte/macrophages. In this regard, Bernard et al. (2)

examined sera from HIV antigen-immunized human volunteers for their effect on the *in vitro* HIV-1 infection of primary human blood monocyte/macrophages. These studies detected only neutralizing antibodies in HIV-immunized individuals. In contrast, Homsy et al. (17) studied sera from HIV-infected humans for their effect on the *in vitro* infection of blood monocyte-derived macrophages and found evidence for an antibody-mediated entry mechanism involving low-affinity Fc receptors (FcR III) and not CD4. This route of virus entry is unique compared with routes reported elsewhere for other primary human monocyte/macrophage populations (15, 33, 34). Although the reason for these differences is unclear, it is possible that either *in vitro* differentiation of these cells by the 12-day culture period or trypsinization prior to HIV-1 infection may have favored the detection of alternative mechanisms of HIV-1 entry. Trypsin is known to cleave the CD4 molecule (20), and *in vitro* culture of adherent blood monocytes has been shown to decrease surface CD4 expression (23) and to affect a variety of other monocyte/macrophage activities (57). Also, the HIV-1 isolates studied on these monocyte-derived macrophages (SF2c, SF33, SF128a, and SF170) were different than those studied here (Ba-L, IIB, D.U. 6587-7, and D.U. 7887-8), thus raising the question that some HIV-1 isolates may be more susceptible to antibody-facilitated entry than others, a possibility suggested previously by Homsy et al. (16). Finally, Perno et al. (34) examined the effect of protein A-purified immunoglobulin G from pooled HIV antibody-positive human sera on the HIV-1 infection of fresh and 5-day-cultured human blood monocytes. With these cells, HIV p24 antigen production was increased over that of controls when very low antibody concentrations (0.05 to 5 ng/ml) were tested with limiting dilutions of virus (<10 minimum infectious doses per well). Other conditions of virus and antibody were consistent with a protective role for antibodies. Importantly, under all antibody and virus conditions examined, the mechanism of virus entry into these primary human monocytes was CD4 dependent.

In conclusion, the mechanism(s) which HIV-1 infects primary human monocytes and macrophages and the role of human antibody in this process are still being analyzed. The results presented here demonstrate that (i) low dilutions of HIV antibody-positive human sera block the HIV-1 infection of freshly isolated primary human blood monocytes and peritoneal macrophages, (ii) HIV-1 infection of these cells is CD4 dependent, and (iii) conditions which enhance the HIV-1 infection of two human cell lines do not facilitate the infection of either primary blood monocytes or peritoneal macrophages by the four virus isolates studied. Although these results are encouraging with regard to the development of HIV-1 vaccines and passive immunotherapies, a number of fundamental issues remain to be clarified, including the *in vitro* effect of antibody on the HIV-1 infection of other primary human cell populations bearing relevant surface antigens and, most importantly, the *in vivo* effect of antibody on HIV-1 infection and disease progression.

ACKNOWLEDGMENTS

We thank Suzie Wortham, Teresa Greenwall, Charlene McDanal, and Annamaria Faust for technical assistance; Barlett Humphries, Anthony Adinolfi, and Karl Blankenship for providing fresh human sera; Raymond Sweet and Martin Rosenberg (Smith Kline & French) for the generous gift of sT4; Ellis Reinherz (Harvard Medical School) for the generous gift of 19Thy5D7; Charles Pegram for chromatographic analysis of 19Thy5D7; S. David Stanley for helpful suggestions; and Liz Ferrell for preparation of the manuscript.

This research was supported in part by Public Health Service grants AI 07922 (P.P.S.), CA 09307, AR 39162, and AI 23308, the Veterans Administration Research Center on AIDS and HIV infection (J.B.W.), and FOD-0758 (T.J.M.).

REFERENCES

- Barnes, D. M. 1988. Another glitch for AIDS vaccines? *Science* 241:533-534.
- Bernard, J., B. Reveil, I. Najman, F. Liautaud-Roger, M. Fouchard, O. Picard, A. Cattan, A. Mabonzo, S. Laverna, R. C. Gallo, and D. Zagury. 1990. Discrimination between protective and enhancing HIV antibodies. *AIDS Res. Hum. Retroviruses* 6:243-249.
- Cheng-Mayer, C., J. Homsy, L. A. Evans, and J. A. Levy. 1988. Identification of human immunodeficiency virus subtypes with distinct patterns of sensitivity to serum neutralization. *Proc. Natl. Acad. Sci. USA* 85:2815-2819.
- Cooper, N. 1987. The complement system, p. 114-127. In D. P. Stites, J. D. Stobo, and J. V. Wells (ed.), *Basic and clinical immunology*, 6th ed. Appleton & Lange, Norwalk, Conn.
- Crowe, S., J. Mills, and M. S. McGrath. 1987. Quantitative immunocytofluorographic analysis of CD4 surface antigen expression and HIV infection of human peripheral blood monocytes-macrophages. *AIDS Res. Hum. Retroviruses* 3:135-145.
- Deen, K. C., J. S. McDougal, R. Inacker, G. Folena-Wasserman, J. Arthos, J. Rosenberg, P. J. Maddon, R. Axel, and R. W. Sweet. 1988. A soluble form of CD4 (T4) protein inhibits AIDS virus infection. *Nature (London)* 331:82-84.
- Desrosiers, R. C., M. S. Wyand, T. Kodama, D. J. Ringler, L. O. Arthur, P. K. Sehgal, N. L. Letvin, N. W. King, and M. D. Daniel. 1989. Vaccine protection against simian immunodeficiency virus infection. *Proc. Natl. Acad. Sci. USA* 86:6353-6357.
- Emini, E. A., P. L. Nara, and W. A. Schleif. 1990. Antibody-mediated in vitro neutralization of human immunodeficiency virus type 1 abolishes infectivity for chimpanzees. *J. Virol.* 64:3674-3678.
- Gardner, M. B. 1989. SIV infected rhesus macaques: an AIDS model for immunoprevention and immunotherapy. *Adv. Exp. Med. Biol.* 251:279-293.
- Gartner, S., P. Markovits, D. M. Markovits, M. H. Kaplan, R. C. Gallo, and M. Popovic. 1986. The role of mononuclear phagocytes in HTLV-III/LAV infection. *Science* 233:215-219.
- Gendelman, H. E., J. M. Orenstein, L. M. Baca, B. Weiser, H. Burger, D. C. Kalter, and M. S. Meltzer. 1989. Editorial review: the macrophage in the persistence and pathogenesis of HIV-1 infection. *AIDS* 3:475-495.
- Gibbs, C. J., C. Mora, R. Peters, F. C. Jensen, D. J. Carlo, and J. Salk. 1989. HIV immunization and challenge of HIV seropositive and seronegative chimpanzees, p. 541-543. In Vth International Conference on AIDS, Montreal, Quebec, Canada.
- Goff, S., P. Traktman, and D. Baltimore. 1981. Isolation and properties of Moloney murine leukemia virus mutants: use of a rapid assay for release of virion reverse transcriptase. *J. Virol.* 38:239-248.
- Gras, G., T. Strub, and D. Dormont. 1988. Antibody-dependent enhancement of HIV-1 infection. *Lancet* i:1285. (Letter.)
- Harbison, M. S., J. M. Gillis, P. Pinkston, R. A. Byrn, R. M. Rose, and S. M. Hammer. 1990. Effects of recombinant soluble CD4 (rCD4) on HIV-1 infection of monocyte/macrophages. *J. Infect. Dis.* 161:1-6.
- Homsy, J., M. Meyer, and J. A. Levy. 1990. Serum enhancement of human immunodeficiency virus (HIV) infection correlates with disease in HIV-infected individuals. *J. Virol.* 64:1437-1440.
- Homsy, J., M. Meyer, M. Tateno, S. Clarkson, and J. A. Levy. 1989. The Fc and not CD4 receptor mediates antibody enhancement of HIV infection in human cells. *Science* 244:1357-1359.
- Homsy, J., M. Tateno, and J. A. Levy. 1988. Antibody-dependent enhancement of HIV infection. *Lancet* i:1285-1286. (Letter.)
- Hu, S. L., P. N. Fultz, H. M. McClure, J. W. Eichberg, E. K. Thomas, J. Earling, M. C. Singhal, S. G. Kosowski, R. B. Swinson, D. C. Anderson, and G. Todero. 1987. Effect of immunization with a vaccine-HIV env recombinant on HIV infection of chimpanzees. *Nature (London)* 328:721-723.
- Ibegbu, C. C., M. S. Kennedy, P. J. Maddon, K. C. Deen, D. Hicks, R. W. Sweet, and J. S. McDougal. 1989. Structural features of CD4 required for binding to HIV. *J. Immunol.* 142:2250-2256.
- Johnson, W. D., B. Mei, and Z. A. Cohn. 1977. The separation, long-term cultivation, and maturation of the human monocyte. *J. Exp. Med.* 146:1613-1626.
- Joualt, T., F. Chapuis, R. Olivier, C. Parravicini, E. Bahraoui, and J. C. Gluckman. 1989. HIV infection of monocytic cells: role of antibody-mediated virus binding to Fc-gamma receptors. *AIDS* 3:125-133.
- Kazazi, F., J. M. Mathijs, P. Foley, and A. L. Cunningham. 1989. Variations in CD4 expression by human monocytes and macrophages and their relationship to infection with the human immunodeficiency virus. *J. Gen. Virol.* 70:2661-2672.
- Lange, J. M. A., D. A. Paul, H. G. Huisman, F. deWolf, H. vandenBerg, R. A. Coutinho, S. A. Danner, J. vanderNoordaa, and J. Goudsmit. 1986. Persistent HIV antigenaemia and decline of HIV core antibodies associated with transition to AIDS. *Br. Med. J.* 293:1459-1462.
- Lasky, L. A., J. E. Groopman, C. W. Fennie, P. M. Benz, D. J. Capon, D. J. Dowbenko, G. R. Nakamura, W. M. Nunes, M. E. Renz, and P. W. Berman. 1986. Neutralization of the AIDS retrovirus by antibodies to a recombinant envelope glycoprotein. *Science* 233:209-212.
- MacKenzie, M. R., C. Zaiss, P. Marx, D. Maul, and N. Lerche. 1989. Prevention of simian AIDS by passive protection, p. 522. In Vth International Conference on AIDS, Montreal, Quebec, Canada.
- Matsuda, S., M. Gidlund, F. Chiodi, A. Cafaro, A. Nygren, B. Morein, K. Nilsson, E. M. Fenyo, and H. Wigzell. 1989. Enhancement of human immunodeficiency virus (HIV) replication in human monocytes by low titers of anti-HIV antibodies in vitro. *Scand. J. Immunol.* 30:425-434.
- Matsushita, S., H. Maeda, Y. Maeda, S. Tokiyoshi, T. Hattori, K. Takatsuki, et al. 1989. Production and characterization of a human-mouse chimeric monoclonal antibody (CB1) against HIV, p. 542. In Vth International Conference on AIDS, Montreal, Quebec, Canada.
- Matthews, T. J., A. J. Langlois, W. G. Robey, N. T. Chang, R. C. Gallo, P. J. Fischinger, and D. P. Bolognesi. 1986. Restricted neutralization of divergent human T-lymphotropic virus type III isolates by antibodies to the major envelope glycoprotein. *Proc. Natl. Acad. Sci. USA* 83:9709-9713.
- McDougal, J. S., M. S. Kennedy, J. K. A. Nicholson, T. J. Spira, H. W. Jaffe, J. E. Kaplan, D. B. Fishbein, P. O'Malley, C. H. Aloisio, C. M. Black, M. Hubbard, and C. B. Reimer. 1987. Antibody response to human immunodeficiency virus in homosexual men: relation of antibody specificity, titer, and isotype to clinical status, severity of immunodeficiency, and disease progression. *J. Clin. Invest.* 80:316-324.
- McKeating, J. A., P. D. Griffiths, and R. A. Weiss. 1990. HIV susceptibility conferred to human fibroblasts by cytomegalovirus-induced Fc receptor. *Nature (London)* 343:659-661.
- Murphey-Corb, M., L. N. Martin, B. Davison-Fairburn, R. C. Montelaro, M. Miller, M. West, S. Ohkawa, G. B. Baskin, J.-Y. Zhang, S. D. Putney, A. C. Allison, and D. A. Eppstein. 1989. A formalin-inactivated whole SIV vaccine confers protection in macaques. *Science* 246:1293-1297.
- Nicholson, J. K. A., G. D. Cross, C. S. Callaway, and J. S. McDougal. 1986. In vitro infection of human monocytes with human T lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV). *J. Immunol.* 137:323-329.
- Perno, C.-F., M. W. Baseler, S. Broder, and R. Yarchoan. 1990. Infection of monocytes by human immunodeficiency virus 1 blocked by inhibitors of CD4-gp120 binding, even in the presence of enhancing antibodies. *J. Exp. Med.* 171:1043-1056.
- Prince, A. M., B. Horowitz, R. W. Shulman, D. Pascual, I. Hewlett, J. Epstein, and J. W. Eichberg. 1990. Apparent prevention of HIV infection by HIV immunoglobulin given prior to low-dose HIV challenge, p. 347-351. In F. Brown, R. Chanock,

- H. Ginsberg, and R. Lerner (ed.), Vaccines 90, Modern approaches to new vaccines including prevention of AIDS. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
36. Prince, A. M., D. Pascual, L. B. Kosolapov, D. Kurokawa, L. Baker, and P. Rubinstein. 1987. Prevalence, clinical significance, and strain specificity of neutralizing antibody to the human immunodeficiency virus. *J. Infect. Dis.* **156**:268–272.
 37. Redfield, R. R., D. C. Wright, and E. C. Tramont. 1986. The Walter Reed staging classification for HTLV-III/LAV infection. *N. Engl. J. Med.* **314**:131–132.
 38. Reynes, M., J. P. Aubert, J. H. M. Cohen, J. Audouin, V. Tricottet, J. Diebold, and M. D. Kazatchkine. 1985. Human follicular dendritic cells express CR1, CR2, and CR3 complement receptor antigens. *J. Immunol.* **135**:2687–2694.
 39. Robert-Guroff, M., M. Brown, and R. C. Gallo. 1985. HTLV-III-neutralizing antibodies in patients with AIDS and AIDS related complex. *Nature* **316**:72–74.
 40. Robinson, W. E., T. Kawamura, M. K. Gorny, D. Lake, J.-Y. Xu, Y. Matsumoto, T. Sugano, Y. Masuho, W. M. Mitchell, E. Hersch, and S. Zolla-Pazner. 1990. Human monoclonal antibodies to the human immunodeficiency virus type 1 (HIV-1) transmembrane glycoprotein enhance HIV-1 infection *in vitro*. *Proc. Natl. Acad. Sci. USA* **87**:3185–3189.
 41. Robinson, W. E., D. C. Montefiori, D. H. Gillespie, and W. M. Mitchell. 1989. Complement-mediated, antibody-dependent enhancement of HIV-1 infection *in vitro* is characterized by increased protein and RNA synthesis and infectious virus release. *J. Acquired Immune Defic. Syndr.* **2**:33–42.
 42. Robinson, W. E., D. C. Montefiori, and W. M. Mitchell. 1987. A human immunodeficiency virus type 1 (HIV-1) infection-enhancing factor in seropositive sera. *Biochem. Biophys. Res. Commun.* **149**:693–699.
 43. Robinson, W. E., D. C. Montefiori, and W. M. Mitchell. 1988. Antibody-dependent enhancement of human immunodeficiency virus type 1 infection. *Lancet* **i**:790–794.
 44. Robinson, W. E., D. C. Montefiori, and W. M. Mitchell. 1988. Will antibody-dependent enhancement of HIV-1 infection be a problem with AIDS vaccines? *Lancet* **i**:830–831. (Letter.)
 45. Robinson, W. E., D. C. Montefiori, W. M. Mitchell, A. M. Prince, H. J. Alter, G. R. Dreesman, and J. W. Eichberg. 1989. Antibody-dependent enhancement of human immunodeficiency virus type 1 (HIV-1) infection *in vitro* by serum from HIV-1 infected and passively-immunized chimpanzees. *Proc. Natl. Acad. Sci. USA* **86**:4710–4714.
 46. Ross, G. D., and M. E. Medof. 1985. Membrane complement receptors specific for bound fragments of C3. *Adv. Immunol.* **37**:217–267.
 47. Sattentau, Q. J., A. G. Dagleish, R. A. Weiss, and P. C. L. Beverley. 1986. Epitopes of the CD4 antigen and HIV infection. *Science* **234**:1120–1123.
 48. Schneider, R. D. 1984. The chemistry and biology of complement receptors. *Springer Semin. Immunopathol.* **7**:221–235.
 49. Sutjipto, S., N. C. Pedersen, C. J. Miller, M. B. Gardner, C. V. Hanson, A. Gettie, M. Jennings, J. Higgins, and P. A. Marx. 1990. Inactivated simian immunodeficiency virus vaccine failed to protect rhesus macaques from intravenous or genital mucosal infection but delayed disease in intravenously exposed animals. *J. Virol.* **64**:2290–2297.
 50. Takeda, A., C. U. Tuazon, and F. A. Ennis. 1988. Antibody-enhanced infection by HIV-1 via Fc receptor-mediated entry. *Science* **242**:580–583.
 51. Unkeless, J. C., E. Scigliano, and V. H. Freedman. 1988. Structure and function of human and murine receptor for IgG. *Annu. Rev. Immunol.* **6**:251–281.
 52. Weinberg, J. B., and A. F. Haney. 1983. Tumor cell killing by human blood monocytes and peritoneal macrophages: lack of alteration by endotoxin or quenchers of oxygen free radicals. *JN CI* **70**:1005–1010.
 53. Weinberg, J. B., M. M. Hobbs, and M. A. Misukonis. 1985. Phenotypic characterization of gamma interferon-induced human monocyte polykaryons. *Blood* **66**:1241–1246.
 54. Weiss, R. A., P. R. Clapham, R. Cheingsong-Popov, A. G. Dagleish, C. A. Carne, I. V. D. Weller, and R. S. Tedder. 1985. Neutralization of human T-lymphotropic virus type III by sera of AIDS and AIDS-risk patients. *Nature (London)* **316**:69–72.
 55. Willey, R., D. H. Smith, L. A. Lasky, T. S. Theodore, R. L. Earl, B. Moss, D. J. Capon, and M. A. Martin. 1988. In vitro mutagenesis identifies a region within the envelope gene of the human immunodeficiency virus that is critical for infectivity. *J. Virol.* **62**:139–147.
 56. Zeira, M., R. A. Byrn, and J. E. Groopman. 1990. Inhibition of serum-enhanced HIV-1 infection of U937 monocytoid cells by recombinant soluble CD4 and anti-CD4 monoclonal antibody. *AIDS Res. Hum. Retroviruses* **6**:629–639.
 57. Zuckerman, S. H., S. K. Ackerman, and S. D. Douglas. 1979. Long-term human peripheral blood monocyte cultures: establishment, metabolism, and morphology of primary human monocyte-macrophage cell cultures. *Immunology* **38**:401–411.