Characterization of Monoclonal Antibodies against the Human Immunodeficiency Virus Matrix Protein, p17^{gag}: Identification of Epitopes Exposed at the Surfaces of Infected Cells†

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Eight monoclonal antibodies reactive with the matrix protein of human immunodeficiency virus type 1 (HIV-1), p17^{gag}, were isolated from rats which had been immunized with solubilized HIV-1 lysate. The epitope specificities of these antibodies were determined with a series of synthetic peptides representing overlapping regions of p17. Six of the antibodies were mapped to three distinct regions of p17, while two antibodies (G11g1 and G11h3) reacted only with intact recombinant p17, suggesting that they were directed against conformational or discontinuous epitopes. All the antibodies bound to HIV-infected cells which had been permeabilized with acetone, but only G11g1 and G11h3 reacted with live HIV-infected cells. Specificity studies with diverse virus strains demonstrated that these two antibodies recognized distinct epitopes, one which was group specific for HIV-1, and one which was shared with HIV type 2 and simian immunodeficiency virus. Binding competition studies indicated that these epitopes were proximal in native p17. Despite their reactivity with intact cells, these two antibodies did not possess appreciable virus-neutralizing activity. These results indicate that a form of p17 is expressed on the surfaces of live HIV-infected cells which is accessible to some, but not all, antibodies against p17. These cell surface molecules may play a role in the generation of antibodies against p17^{gag} that are characteristic of early stages of HIV infection, and they may act as natural targets for the immune system and as potential targets for immunotherapy of HIV-infected cells.

The N-terminal segment of the human immunodeficiency virus (HIV) *gag* protein, p17, is myristylated at its amino terminus and is believed to function as a matrix protein in the assembly of viral cores at the plasma membrane (13, 28). It has been reported that some antibodies directed against p17^{gag} possess virus-neutralizing activities (14, 22), suggesting that p17 is exposed on the surfaces of virions and that it plays a critical role in viral infection. This is a surprising result, since it is generally believed that the only virion proteins exposed on the surfaces of retroviral particles are the envelope proteins, whereas the core proteins are localized to the interior of the viral membrane. Consistent with this are immunoelectron microscopy and computer modeling studies of HIV which suggest that p17 is localized to the interior surface of the viral membrane via its myristic acid substituent (2, 12).

To examine the localization and function of p17, we generated a series of monoclonal antibodies against p17^{gag} and characterized these antibodies for epitope specificity and conservedness, reactivity with live HIV-infected cells, and ability to neutralize HIV infectivity. Two of the antibodies appeared to recognize nonlinear epitopes. These epitopes were exposed on the surfaces of intact infected cells.

MATERIALS AND METHODS

Isolation of monoclonal antibodies. Fisher rats were immunized subcutaneously with disrupted preparations of HIV type 1 (HIV-1) strain HTLV-IIIb (50 µg/ml) prepared as

emulsions with complete Freund's adjuvant, followed by monthly boosters with an equivalent amount of virus in incomplete Freund's adjuvant. Once a high titer of antibodies against HIV-1 proteins was obtained (after three or four immunizations), spleen cells of the immunized animals were fused with SP2/0 myeloma cells, and supernatants of hybridoma clones were screened by enzyme-linked immunosorbent assay (ELISA) on plates coated with HIV lysates. Positive wells were recloned to obtain stable monoclonal antibody-producing hybridoma lines.

Epitope mapping. The epitope specificities of the antibodies against p17 were determined by a peptide ELISA. Titertek immunoassay plates (Flow-ICN Inc.) were coated with a series of overlapping peptides representing the complete HTLV-IIIb p17 sequence (19). Solutions of the peptides at 1 µg/ml in 15 mM Na₂CO₃–35 mM NaHCO₃, (pH 9.6) were added to individual wells and incubated overnight. The wells were then blocked with 2% bovine serum albumin and washed with phosphate-buffered saline (PBS) containing 0.05% Tween 20. Antibodies at 1 µg per well in PBS were incubated with the peptide-coated wells for 1 h, and after being washed, the wells were incubated with alkaline phosphatase-conjugated rabbit anti-rat immunoglobulin (Ig) for 30 min. Bound antibody was quantitated by adding *p*-nitrophenyl phosphate in diethanolamine buffer, and after incubation for 30 min, by determining absorbance at 405 nm on a Titertek Multiskan Plus ELISA reader. All incubations were performed at room temperature. Synthetic peptides were purchased from the Peptide Laboratory Inc., Berkeley, Calif.

Immunoaffinity purification of antibodies. Recombinant p17 (rp17) synthesized in *Escherichia coli* and purified to homogeneity was provided by Transgene. AH-Sepharose 4B (Pharmacia) was activated by treatment with an equal vol-

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ume of 25% glutaraldehyde in 0.5 M sodium carbonate buffer (pH 9.3). The activated gel (0.25 ml) was coupled to 1 mg of rp17 in 1 ml of the same buffer, and excess sites on the beads were blocked by the addition of 1 ml of 100 mM ethanolamine, pH 7.5. Antibodies were affinity purified by passing 50 ml of supernatant medium of the hybridoma cultures over the column five times, removing unbound material by washing with PBS, and eluting specifically bound antibodies with 1 ml of 0.1 M glycine buffer, pH 2.7. The eluants were immediately neutralized with 0.15 ml of 1 M Tris buffer (pH 8.0), and the antibody solutions were dialyzed against PBS. When necessary, the antibodies were concentrated by centrifugation on Centricon spin columns. Antibody purity was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and protein concentrations were estimated by determining the optical density at 280 nm or by the Bradford dye-binding assay, using purified rat IgG as the standard. Antibody isotypes were determined by radial immunodiffusion assays specific for rat IgGs.

Specificities of monoclonal antibodies. The protein specificities of the antibodies were determined by Western blot (immunoblot) analyses, using commercial HIV-1 Western blot kits (Du Pont). The strips were incubated with a 1:100 dilution of an HIV-1-seropositive human serum or with purified monoclonal antibodies (5 µg/ml) and developed as previously described (17). The reactivities of antibodies against different HIV-1 isolates were tested by immunofluorescence assays against H9 cells infected with the isolate in question. Approximately 35,000 infected cells were allowed to absorb to 5-mm multiwell microscope slides (Cell-Line Associates, Inc.) which had been coated with polylysine (100 µg/ml). After fixation with acetone, the cells were stained by treatment with monoclonal antibodies and then treatment with a fluorescein isothiocyanate (FITC)-conjugated goat anti-rat Ig second antibody. All antibody solutions were prepared in PBS containing 1 mg of bovine gamma globulin (ICN Immunobiologicals Inc.) per ml to reduce nonspecific binding. Under these conditions, no background fluorescence was detected for any of the antibodies in uninfected H9 cells. Immunofluorescence was visualized with a Nikon Diaphot microscope equipped for epifluorescence and automatic exposure and was recorded photographically with a mounted Nikon camera, using Ektar 25 film and Kodak processing.

Competition binding studies. Affinity-purified G11h3 antibody was biotinylated as described previously (7) with biotinamidocaproate *N*-hydroxysuccinimide (Sigma). Purified rp17 was bound to 96-well plates at a concentration of 100 ng per well and reacted with 50 ng of the biotinylated antibody in the presence of various amounts of unlabeled antibodies. Binding of the biotinylated antibody was quantitated by the addition of streptavidin conjugated to alkaline phosphatase (Vector ABC kit) and then *p*-nitrophenyl phosphate substrate.

Identification of cell surface epitopes. A live-cell immunofluorescence assay was used to determine whether any of the antigens recognized by the anti-p17 monoclonal antibodies were expressed on the surfaces of live cells. Intact HTLV-IIIb-infected H9 cells were mixed with monoclonal antibodies and allowed to attach to polylysine-coated multiwell slides for 30 min at 37°C. The slides were then washed three times with PBS before fixation of the cells with acetone. Bound antibodies were visualized by treatment with FITC-conjugated second antibody, as described above. Antibodies against known internal antigens did not bind to cells under these conditions.

HIV neutralization assays. The ability of the anti-p17 monoclonal antibodies to neutralize HIV-1 infection was determined with two different concentrations of virus. A 24-h quantitative fluorescent focus assay was used to measure infection by a high-titered stock of virus after a single round of replication. Supernatant medium of HTLV-IIIb-infected cells was filtered through a 0.8-µm-pore-size Nalgene syringe filter and diluted with fresh culture medium (RPMI 1640 plus Pen-Strep plus 10% fetal bovine serum) to a concentration of approximately 4×10^4 infectious units of virus per ml (as determined by limiting dilution assays). A 50-µl sample of virus was preincubated for 30 min at room temperature with an equal volume of purified antibody diluted into fresh medium, and the mixture was then added to 100 µl of medium containing approximately 100,000 uninfected H9 cells in wells of 96-well tissue culture plates. After incubation for 20 to 24 h at 37°C, 35 µl of the cell suspension was allowed to absorb to separate wells of polylysine-coated slides for approximately 15 min, washed by successive dipping in two baths of PBS and one of distilled water, and fixed by incubation in acetone for 8 min. Cells were tested for infection by staining sequentially with a rat monospecific anti-*nef* serum, prepared in this laboratory, and then with FITC-conjugated rabbit anti-rat Ig serum (Zymed). The slides were then counterstained with Evans blue, and the extent of infection was quantitated by counting immunofluorescent cells versus total cells, using a Nikon Diaphot microscope equipped for epifluorescence. A minimum of 2,000 cells per well were counted, and each sample was assayed in duplicate. Virus neutralizations were also done with a diluted virus solution (1,000 infectious units per ml). Cells were incubated with virus plus antibodies for 1 h at 37°C, the virus-containing medium was removed, and the cells were cultured in the continuous presence of antibody. Wells were scored for infection after 6 days by immunofluorescence, as described above.

Virus isolates were obtained from the following sources: HTLV-IIIb and SF-2 strains from J. Laurence at Cornell University Medical School, MN and RF from the NIH AIDS Research and Reference Reagent Program, the ROD strain of lymphadenopathy-associated virus type 2 from L. Montagnier at Institut Pasteur, and the BK28 strain of simian immunodeficiency virus (SIV) from Phyllis Kanki at Harvard University.

RESULTS

Characterization of monoclonal antibodies. Nine monoclonal antibodies were isolated from two separate fusions of spleen cells of rats immunized with solubilized HTLV-IIIb lysate (Table 1). Screening was initially performed by ELISAs against the same viral lysate used as the immunogen, and specificity was determined by Western blot assays, using commercial strips prepared with lysates of the same strain of HIV-1 (Fig. 1). A human immune serum recognized a number of viral proteins, including components of 160 and 120 kDa, reverse transcriptase (p66 and p51), the *gag* polyprotein (p55), gp41, integrase (p31), and *gag* proteins p24 and p17. We have previously shown that the 160- and 120-kDa bands consist mostly of gp41 trimers and tetramers (17). Eight of the monoclonal antibodies reacted strongly with the p17 band, and these antibodies also recognized the *gag* precursor protein, p55. Several antibodies also reacted with a larger component of approximately 80 to 90 kDa, which may represent a processing intermediate of the *gag-pol* polyprotein. One antibody recognized p24 (lane J). No

TABLE 1. Summary of the properties of the rat monoclonal antibodies described in this report^a

Antibody	Isotype	Antigen	WB	RIP	Live-cell IF	IIIb	MN	SF-2	LAV-2	SIV
12A-G11g1	IgG2a	p17	+	+	+	+	+	+	+	+
12H-G11h3	IgG2a	p17	+	+	+	+	+	+	—	—
12H-D3b3	IgG2a	p17	+	+	—	+	+	+	—	—
10A-B4f8	IgG2a	p17	+	+	—	+	+	+	—	—
12G-A8g2	IgG2a	p17	+	+	—	+	+	+	—	—
12G-D7h11	IgG2a	p17	+	+	—	*	*	*	*	*
12I-D12g2	IgG2a	p17	+	+	—	*	*	*	*	*
12G-H1c7	IgG	p17	+	+	—	+	+	+	—	—
12A-E9a10	IgG2a	p24	+	+	—	+	+	+	+	+

^a WB, Western blot activity; RIP, radioimmunoprecipitation against radiolabeled p17; Live-cell IF, immunofluorescence against live intact infected H9 cells. All these assays were performed for the HTLV-IIIb strain of HIV-1. Reactivities against the other virus strains listed in the table (MN, SF-2, LAV-2, SIV) were determined by immunofluorescence assays against H9 cells infected with the appropriate viral isolates, assayed after acetone fixation. *, reactivity against uninfected H9 cells and thus an indeterminate phenotype.

bands were seen in a control sample in which the first antibody was omitted. Similar specificities were observed when radioimmunoprecipitation assays were performed, using virus labeled either in vivo with ³⁵S-labeled amino acids or in vitro with ¹²⁵I (16).

The strain specificity of these antibodies was determined by immunofluorescence assays of the reactivity of the antibodies with cells infected by various strains of HIV (Table 1). Seven of eight anti-p17 antibodies reacted with three divergent strains of HIV-1, MN, IIIb, and SF-2, but not with HIV type 2 (HIV-2) or SIV. Thus, it appears that these antibodies react with conserved, group-specific epitopes. The p17 epitope recognized by antibody G11g1 and the p24 epitope recognized by antibody E9a10 were also conserved across viruses of different types and host species. These antibodies cross-reacted with cells infected with the ROD strain of HIV-2 as well as with the BK28 strain of SIV and thus appear to recognize interspecies-specific epitopes.

Epitope mapping. The epitope specificities of the monoclonal antibodies against p17 were investigated by examining the ability of these antibodies to bind to synthetic peptides corresponding to various regions of the p17 sequence. A

series of 13 overlapping peptides ranging in size from 11 to 16 amino acids which covered the entire p17 sequence were utilized (Fig. 2). An additional larger peptide (labeled 9A in Fig. 2 and 86-115 in Fig. 3) which overlapped with regions of the smaller peptides 9, 10, and 11 was also tested. Reactivity of the monoclonal antibodies against each of these peptides was measured in an ELISA format, with rp17 also assayed in parallel (Fig. 3). All the antibodies bound strongly to rp17. Antibody B4f8 bound to peptide 6, and antibody D3b3 reacted with peptide 7, localizing the epitopes recognized by these two antibodies to sequences present in these peptides. Four of the antibodies, A8g2, D7h11, D12g2, and H1c7, reacted with peptide 9A but not with smaller peptides 9, 10, and 11 which contained different regions of the larger peptide. This suggests that the epitope(s) recognized by these four antibodies corresponds to sequences divided between peptides 9 and 10 or peptides 10 and 11. Alternatively, some of these epitopes may be conformational, involving sequences present at the two ends of peptide 9A. Antibodies G11g1 and G11h3 bound well to rp17, but they did not bind to any of the peptides tested. A similar lack of reactivity for these two antibodies was also observed when a different set of larger peptides was tested (data not shown). This suggests that they recognize a discontinuous sequence or a conformational structure dependent on distant regions of the protein.

To obtain further information about the epitopes recognized by antibodies G11g1 and G11h3, we performed reciprocal binding competition studies. These two antibodies were biotinylated, and the ability of each of the other anti-p17 monoclonal antibodies to compete with the binding of the labeled antibodies to rp17 was determined. As shown in Fig. 4, binding of antibody G11h3 was inhibited well by antibody G11g1 but not appreciably by the other antibodies even at the highest concentration tested. Similar results were obtained when biotinylated G11g1 was competed for by excess G11h3 (data not shown). These results indicate that the epitopes recognized by G11g1 and G11h3 are proximally located in the p17 molecule and are separated from the epitopes recognized by the other antibodies tested.

Cell surface staining. To examine the localization of molecules carrying p17 epitopes in infected cells, we tested the ability of the monoclonal antibodies described above to react with live cells. Live HTLV-IIIb-infected cells were incubated with the monoclonal antibodies in complete growth medium, and the cells were allowed to attach to polylysine-coated slides. Unbound antibodies were washed away, with care taken to prevent dehydration of cells. The cells were

FIG. 1. Western blot analysis of monoclonal antibodies (MAbs). The following samples were analyzed against HIV-1 lysate: lane A, human immune serum; lane B, MAb G11h3; lane C, MAb G11g1; lane D, MAb D3b3; lane E, MAb B4f8; lane F, MAb D12g2; lane G, MAb D7h11; lane H, MAb A8g2; lane I, MAb H1c7; lane J, MAb E9a10; lane K, control (no first antibody).

FIG. 2. Peptides used for epitope mapping of monoclonal antibodies against HIV-1 p17. The p17 sequence was derived from the BH10 clone strain of HIV-1 (19) and is shown using the single-letter code. Acidic and basic residues are indicated by + and -, respectively, under the residue. The numbering system refers to the bacterially synthesized recombinant molecule and designates the amino-terminal methionine as residue 1.

then fixed in acetone and stained with an FITC-conjugated second antibody to visualize bound antibodies. Under these conditions, antibodies G11g1 and G11h3 stained the cells (Fig. 5). The staining pattern observed appeared to be localized to surface membranes, in contrast to a more generalized cytoplasmic staining observed for cells previously permeabilized with acetone. None of these antibodies stained uninfected H9 cells, whether the antibodies were added before or after acetone fixation. The remaining anti-p17 antibodies and antibodies against other *gag* proteins did not react with live cells (data not shown), although all these antibodies efficiently stained infected cells which had been fixed with acetone prior to addition of the first antibody. This demonstrates that the epitopes recognized by antibodies G11g1 and G11h3 are exposed on the surface of live HIV-infected cells.

Virus neutralizations. Previous reports have described antisera and monoclonal antibodies directed against HIV p17 which possess virus-neutralizing activity (14, 22). Since monoclonal antibodies G11g1 and G11h3 reacted with a cell surface component of infected cells, it was of interest to determine whether these antibodies also neutralized viral infectivity. Neutralization of the HTLV-IIIb strain of HIV was measured by a quantitative fluorescent focus assay. As controls, a p17 antibody negative for intact cells (D3b3) and a human monoclonal antibody specific for the CD-4 binding site of gp120 (C7.3c [25a]) were also assayed (Fig. 6). Whereas the anti-gp120 antibody reduced infection with 4×10^4 infectious units per ml by greater than 80% at concentrations below 1 μ g/ml and greater than 95% at 10 μ g/ml, significant neutralization was not seen for either of the anti-p17 antibodies even at a concentration of 100 μ g/ml. To confirm the lack of neutralizing activity by the anti-p17 antibodies, we performed a neutralization experiment with 100 infectious units of virus. Under these conditions, 100% of the cells were infected by 6 days postinfection in control wells and in wells containing 100 μ g of antibody G11h3 per ml, whereas no infection was observed in wells which received 10 μ g of the anti-gp120 antibody per ml. These results suggest either that the anti-p17 antibodies do not bind

to the surface of infectious virions or that if they do, such binding is not detrimental to functions of the virus required for infection.

DISCUSSION

This report describes nine monoclonal rat IgG antibodies against the HIV *gag* gene, eight which are specific for p17 and one which is specific for p24. Seven of the anti-p17 antibodies are group specific for HIV-1, in that they reacted with three different HIV-1 strains, HTLV-IIIb, SF2, and MN, but not with HIV-2 or SIV isolates. One of the anti-p17 antibodies (G11g1) and the anti-p24 antibody (E9a10) recognize highly conserved, interspecies-specific epitopes, as they reacted strongly with HIV-2 and SIV isolates in addition to the three HIV-1 isolates. All these antibodies reacted in a number of different assays, including ELISA, Western blotting, and radioimmunoprecipitations, and most of the antibodies worked well in immunofluorescence assays (two of the anti-p17 antibodies gave background fluorescence on uninfected cells [Table 1]). Thus, these antibodies are potentially useful for a broad range of applications.

It has been proposed that a portion of p17 may be exposed at the surfaces of virions and infected cells and thus may serve as a target of neutralizing antibodies (14, 22). To test whether the epitopes recognized by the antibodies described in this report were exposed on the cell surface, we used a live-cell immunofluorescence assay. These experiments demonstrated that two of these antibodies (12A-G11g1 and 12H-G11h3) reacted strongly with live cells. Despite this reactivity, these antibodies did not possess any measurable virus-neutralizing activities at concentrations as high as 100 μ g/ml. Studies to ascertain directly whether these antibodies also bound to the surface of intact virions were not conclusive (data not shown). It is possible that the antigen recognized by these antibodies is exposed only on the cell surface but not in virions. Alternatively, the antigen may be exposed on the virion surface, but since it is not directly involved in the infectious process, antibodies against it are not neutralizing. An example of this phenomenon is the lack of

FIG. 3. Epitope mapping of monoclonal antibodies to p17. ELISAs were used to measure binding of the antibodies against plates coated with rp17 and peptides described in the legend to Fig. 2. Data are plotted as optical density at 405 nm for each peptide. Reactivities greater than 15% of that obtained with rp17 were considered positive.

neutralization exhibited by antibodies against regions of the transmembrane proteins of murine leukemia virus and HIV (6, 21).

The epitopes recognized by six of the anti-p17 antibodies described in this report were localized by mapping studies with synthetic peptides, whereas antibodies G11g1 and

Ratio of competing antibodies to biotinylated antibody

FIG. 4. Competition of binding of biotinylated monoclonal antibody G11h3 to rp17-coated plates. A 50-ng sample of the biotinylated antibody was mixed with increasing amounts of unlabeled antibodies, and the extent of binding was measured in an ELISA with alkaline phosphatase-labeled avidin. Data are plotted as the percentage of optical density at 405 nm versus a control in which no competing antibodies were added. □, G11h3; ♦, G11g1; ■, D3b3; ♦, B4f8; ■, A8g2; □, D12g2; ▲, D7h11; ▲, H1c7.

G11h3 reacted only with intact p17 and thus appear to recognize discontinuous epitopes. Previous studies have identified epitopes on p17 reactive with mouse monoclonal antibodies. Papsidero et al. (14) mapped a neutralizing antibody to the sequence ¹⁷EKIRLR present in peptide 2 (Table 1). They also identified a second antibody with neutralizing activity which reacted with two discontinuous peptides, ¹²ELDRWEKIRLR in peptide 2 and ¹⁰⁰ALDKIE, present in peptides 9A and 10. The basis for the neutralizing activities of these antibodies is not clear. Hinkula et al. (8) also mapped a purported conformational epitope to the sequences ²³PGGKK present in peptide 3 and ¹²⁸VSQNY present in peptide 13. In both of the above cases, these antibodies reacted with peptides containing either of the two discontinuous sequences and thus are distinct from antibodies G11g1 and G11h3 described in this report.

Several other antibodies have been mapped to the carboxy terminus of the molecule. Niedrig et al. (11) identified the sequence ¹¹³KKAQQAADT present in peptide 12 as an epitope, while Cogniaux et al. (3) mapped epitopes to the sequences ¹⁰⁰ALDKIEEQNKSKKKAQQAADT, which overlaps peptides 10, 11, and 12, and ¹¹⁸AAADTGHSSQV SQNYPVQN, which is related to peptides 12 and 13. Ferns et al. (5) isolated four monoclonal antibodies reactive with a peptide from the C terminus of SF2 p17, ¹¹⁹AAGTGNS SQVSQNY, which is related to peptide 13, and one antibody directed against the sequence ¹⁰¹LEIKIEEQNKSKKKA, which is similar to sequences in peptides 10 and 11. Some of these epitopes may be related to those recognized by the four antibodies described in this report which bind to peptide 9A (corresponding to amino acids 86 to 115).

Peptide 9A (called HGP-30 in references 10 and 22) contains a region with sequence similarity to the thymic hormone thymosin α1, and antisera against thymosin have been reported to cross-react with p17 and to neutralize HIV infectivity. Although the immunological relatedness between p17 and thymosin was not confirmed by others (20), this

12H-G11H3 vs. HTLV-IIIb/H9 (Fixed cells)

12H-G11H3 vs. HTLV-IIIb/H9 (Live cells)

12H-G11H3 vs. uninfected H9 (Fixed cells)

12H-G11H3 vs. uninfected H9 (Live cells)

FIG. 5. Immunofluorescent visualization of binding of monoclonal antibody G11h3 to live (right panels) and acetone-fixed (left panels) HIV-infected cells (top panels). Results with uninfected H9 controls are shown in the bottom panels. Cells were counterstained with Evans blue and appear red. Bound antibody was visualized with an FITC-conjugated second antibody and results in green fluorescence.

peptide was recently shown to be recognized by cytotoxic lymphocytes from HIV-infected individuals (1). It will be of interest to determine the possible interaction of thymosin with the four antibodies reactive with peptide 9A described in this report and to assay these antibodies for cross-competition with other monoclonal antibodies which have been shown to be directed against this region.

The nature of the cell surface molecule carrying the epitopes recognized by antibodies G11g1 and G11h3 was not resolved in the present study. Studies with murine leukemia viruses have also shown the presence of *gag* products on the cell surface (15, 23, 26, 27). These do not, however, correspond to the *gag* proteins found packaged in viral particles but represent products of an alternate biosynthetic pathway in which the *gag* gene is expressed as a glycosylated polypeptide containing a hydrophobic leader sequence (4, 18, 24). The molecule synthesized by endogenous ecotropic murine leukemia viruses is referred to as the Gross cell surface antigen and has been shown to function as an efficient target for antibody- and cell-mediated cytotoxicity reactions (9, 25). We have recently obtained information suggesting that similar molecules are synthesized in HIV-infected cells and may account for the cell surface reactivities described in this report (unpublished data).

FIG. 6. Neutralization of HTLV-IIIb infection by monoclonal antibodies. The number of cells infected after 24 h was counted, converted to a percentage of the control infection in the absence of any antibodies, and plotted versus the concentration of the monoclonal antibody present during the infection. $\circ$, C7.3c; $\square$, G11g1; $\triangle$, G11h3; $\blacksquare$, D3b3.

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