JOURNAL OF VIROLOGY, Dec. 1983, p. 697–708
0022-538X/83/120697-12$02.00/0

Vol. 48, No. 3

Cell Receptors for Baboon Endogenous Virus Recognized by Monoclonal Antibodies

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Received 17 August 1982/Accepted 1 September 1983

Hybridomas from mice immunized with baboon endogenous virus (BaEV) from A204(M7) cells produced several antiviral monoclonal antibodies and, in addition, antibodies D-12 and E-4, which appeared to be virus specific because they reacted with BaEV but not with Mason-Pfizer virus or RD-114 virus. However, they also bound to human virus-free cells, and they did not recognize BaEV from bat or canine host cells. Cell membrane targets for these antibodies comigrated with an 18,000-dalton protein, which may contain specific determinants of BaEV receptors since antibody masking of these cell sites prevented BaEV but not Mason-Pfizer virus or RD-114 virus adsorption. However, RD-114 virus interfered with BaEV adsorption. Thus, the two viral receptors must be adjacent, but the antibody D-12 and E-4 targets are not within the active site of RD-114 virus receptor. Conversely, cell coating with BaEV from bat or canine hosts inhibited antibody D-12 binding. Noncultivated human lymphocytes and cells from fetal organs bound much less antibody D-12 than did cells from established cell lines, with a correlation between amounts of antibody D-12 acceptor sites and BaEV receptors. Thus, in vivo, BaEV infection of human cells may be inefficient. In vitro, antibody D-12 treatment of chronically infected A204(M7) cells caused intracellular accumulation of viral proteins and decreased virus release, with no such effect on RD-114 virus-producing cells. Canine cells bound antibody D-12 only if coated with BaEV from A204(M7) cells, indicating that the human determinant coadsorbed with the virions to animal cells. Possibly, determinants of cell receptors participate in BaEV maturation and become associated with the virions.

We previously immunized mice with semipurified baboon endogenous virus (BaEV) which had been synthesized in the human chronically infected A204(M7) cell line. We prepared monoclonal antibodies A-6 and C-9, which neutralize BaEV and are directed at two very close or similar determinants of the glycoprotein (gp) 70, whereas the nonneutralizing antibody B-9 corresponds to another gp epitope. Antibody F-12 is directed at an 18,000-dalton protein detected in disrupted virions (5). We found that the latter antibody also reacted with a similar polypeptide of RD-114 virus, whereas the anti-gp antibodies were strictly BaEV specific. In addition, we selected antibodies D-12 and E-4, which were not virus specific since they reacted with virus-free cells. However, in a solid-phase radioimmunoassay, these antibodies bound strongly to semipurified BaEV. This indicated either that semipurified virus particles used for mouse immunization and in the radioimmunoassay contained random host cell contaminants or that a more specific host cell determinant is physiologically incorporated within the virus particle, e.g., at the time of maturation. We show that the cell sites recognized by antibodies D-12 and E-4 are two neighboring determinants which are located within a specific receptor for BaEV only present in human cells, and we discuss the possibility that portions of the cell receptor may be integrated within the virus particles or at least firmly attached to them. The antibodies to BaEV receptors are also useful for studying the distribution of these specific receptors in various human tissues and cell lines.

MATERIALS AND METHODS

Viruses, cells, and antibodies. Five cell lines chronically infected with retroviruses were used in this study. The human A204(M7) and the bat CCL-88-M7 cells produced the M7 strain of BaEV, the dog thymus Fcf2th-BILN cells produced the BILN strain of this virus, the human RD-114 cells produced the feline RD-114 virus, and clone 7 of the monkey foreskin cells

940C3 synthesized Mason-Pfizer virus (MP-MV). Virus-free corresponding cells were available for the first four lines cited, but not for the 940C3 line. Vero cells were used as control virus-free monkey cells. The biological activity of viruses in the cell supernatants was quantitated by the number of syncytia formed by BaEV on RSa cells and by RD-114 virus on RSa or KC cells (5, 17). Other cell lines were those routinely used in many laboratories, including the diploid human fibroblasts MRC5, the human B lymphoblast cell lines NC37 and Raji, the T lymphoblast Molt line, the mouse cells L929, and the rat cells NRK. Suspensions of peripheral blood lymphocytes and of fetal cells as well as of syncytiotrophoblastic cells from human term placenta were prepared as described previously (18). Monoclonal antibodies A-6, C-9, and B-9 directed against the gp70 of BaEV, F-12 directed at an internal viral polypeptide of 18,000 daltons, and the antibodies D-12 and E-4 directed against host cell components were prepared in this laboratory. Mas 1532 and Mas 018b monoclonal antibodies were bought from Sera-Lab Ltd., Crawley Down, Sussex, England. The first antibody was provided in the ascite form and is an immunoglobulin G2a (IgG2a) specific for the 43,000-dalton gp chain of the HLA-A, -B, and -C antigens, and the second antibody was the supernatant from hybridoma cultures and is an IgG2a directed against β_2 microglobulin. Antisera against HeLa and NC37 cells were from rabbits immunized in our laboratory.

Antibody binding to cells and viral antigens. The ability of cells to adsorb immunoglobulins of IgG2a and IgM classes was monitored by a subsequent treatment with IgG directed to mouse IgG2a or IgM and conjugated with fluorescein isothiocyanate from Nordic Immunological Laboratories and analysis of cell fluorescence by flow cytofluorometry as described previously (5). Mean fluorescence intensity per cell (ϕ) was determined according to the following formula:

$$\phi = 1/cN \sum_{i=1}^{c} in_i$$

where c is the number of channels of the pulse-height analyzer, N is the total cell number, i is the channel number of the pulse-height analyzer, and n_i is the number of cells in channel i. A blank value, ϕ_A representing the cellular autofluorescence was routinely subtracted from each reading. The level of background fluorescence (ϕ_F) was determined by analyzing appropriate negative controls such as cells labeled with the fluorescein isothiocyanate conjugate alone. This method could not be applied to cells greater than 50 μm in diameter, such as the syncytiotrophoblastic cells used in part of this study. Binding of immunoglobulin of the IgG class was also studied by measuring the amount of ^{125}I-labeled protein A that antibody-treated cells could adsorb. For this assay, 50-μl samples of phosphate-buffered saline with 2% glucose (pH 7.2) (PBS-glucose) containing 5×10^4 cells were distributed into flexible polyvinyl chloride microtiter plates with 96 U-shaped wells (Falcon Plastics, Oxnard, Calif., 3911-III). The plates were centrifuged for 5 min at 170 × g in a Labofuge II Heraeus-Christ centrifuge. Monoclonal immunoglobulins (50 μl) diluted in PBS-glucose with 1% bovine serum albumin (BSA) and 0.02% sodium azide were added per well and left in contact for 1 h at 37°C and then 1 h at 4°C. Nonadsorbed immunoglobulins were removed after plate centrifugation at 540 × g, followed by two washings with 150 μl of 1% BSA in PBS and intervening centrifugations. ^{125}I-labeled protein A prepared each month in this laboratory by the chloramine T technique was diluted in PBS with 0.5% ovalbumin, and 3×10^4 cpm were distributed per well. After 30 min at 37°C and three rounds of washes and centrifugations, the cell pellets were left to dry, the wells were cut, and radioactivity was counted in a Berthold gamma spectrometer.

When disrupted cells, virions, or soluble proteins were assayed for antibody binding, a similar procedure was applied, except that the microtiter plates were left to dry for 24 h at 37°C after distribution of the antigens into the wells. After drying, each well was treated with 50 μl of 5% BSA for 1 h at 37°C, and BSA was discarded before addition of the antibodies.

When the antibodies tested were of the IgM class, an intermediate incubation with a 1:500 dilution of rabbit anti-mouse IgM serum was performed before addition of ^{125}I-labeled protein A.

Serological detection on nitrocellulose. (i) Antigens. Baboon endogenous virus (strain M7) was concentrated from the culture supernatant of A204- or CCL-88-virus producing cells as described previously (5) and purified by isopycnic centrifugation on a continuous 4 to 36% metrizamide gradient at 110,000 × g for 4 h. Cellular membranes (9) were prepared from monolayers of control or M7-producing A204 human cells and CCL-88 bat cells. The preparation of the P50 fraction was carried out as described by Garber et al. (7), except that dithiothreitol was omitted from the homogenizing buffer. The protein concentration of these antigens, suspended in 10 mM Tris-hydrochloride (pH 7.4)–10^{-4} M phenylmethylsulfonyl fluoride–100 U of Trasylol per ml, was determined by the method of Bradford (2).

(ii) Electrophoresis and transfer. Samples (350 μg) from cell membranes and 50 μg of viral proteins were denatured at 100°C for 2 min in sodium dodecyl sulfate (1% sodium dodecyl sulfate–10% glycerol in 0.2 M Tris-hydrochloride, pH 6.8) and submitted to electrophoresis in 10 or 13% acrylamide gels. Sets of three different antigens were run in parallel. These were the P50 fraction from virus-producing cells, the P50 fraction from control cells, and the virus produced by those cells. Molecular weight markers (^{14}C-protein mixture from the Radiochemical Centre, Amersham, England) were run in a separate lane. Immediately after electrophoresis, the polypeptides were transferred to nitrocellulose paper as described by Towbin et al. (19) in a Pharmacia destainer GE 4 at 24 V for 2.5 h at 4°C.

(iii) Immunological reactions. The nitrocellulose paper was saturated overnight at 4°C in 2% BSA–0.05% Nonidet P-40 in TNE buffer as described by Portis and McAtee (13). The sheet was cut into three identical pieces containing three lanes each. The nitrocellulose pieces were incubated with the different monoclonal antibodies (culture medium diluted 1:2 in TNE buffer containing 0.05% Nonidet P-40 and 0.1% gelatin) for 4 h on a rocking platform. After repeated washings in the same buffer, the antibody complexes were detected by incubation with ^{125}I-labeled protein A (4×10^5 cpm/ml, 6 ml for each nitrocellulose piece of 27 cm^2),

followed by autoradiography. An additional incubation of 1 h with a 1:2,000 dilution of rabbit anti-mouse IgM serum (Nordic Laboratories) was carried out before protein A addition, to localize the IgM antibody E-4.

Assay for virus adsorption. Two assays were used. In the first assay, 1 ml of supernatant from 3-day-old cell cultures producing MP-MV, BaEV, or RD-114 virus was mixed with a pellet containing 2×10^7 cells from the culture which was assayed for the presence of virus receptors. After 1 h at 37°C, cells were spun and nonadsorbed virus remaining in the supernatant was counted by distributing 200-μl samples into six wells containing nonconfluent RSa cells which had been plated 24 h before. Syncytia were counted 48 h later, as described previously (5). In the second assay, 100 ml of culture medium from BaEV-producing cells, containing about 1,000 syncytium-forming units per ml, were ultracentrifuged, and the virus pellet was suspended in 3 ml of medium and mixed with 2×10^6 cells whose virus receptors were being studied. After 1 h at 4°C, the cells were washed and samples of 10^5 cells were distributed into four wells. The amount of bound virus was then measured by sequential treatments with saturating amounts of monoclonal antibody A-6, directed at the gp of BaEV, and ^{125}I-labeled protein A, as described above.

RESULTS

Antibody binding to viruses and cells. Purified and disrupted virions of BaEV(M7) grown in A204 and in CCL-88 cells, as well as virions of RD-114 virus, were sequentially treated with monoclonal antibodies and ^{125}I-labeled protein A (Table 1). Antibody F-12 recognized a component present in both BaEV and RD-114 virus, but only BaEV bound anti-gp antibodies C-9 and A-6 as well as antibodies D-12 and E-4. However, the two latter antibodies only bound to BaEV(M7) grown in A204 cells, whereas the reaction of the other antibodies was independent of the host cell origin of the virus. Like BaEV(M7) of bat cell origin, the BILN strain of BaEV produced by the canine FCf2th cells only reacted with antiviral antibodies A-6, C-9, and F-12 but not with antibodies D-12 and E-4 (data not shown). Since this indicated that antibodies D-12 and E-4 might recognize a human host cell component, their binding to cells of human and animal lines was studied. Reaction with the human cells of HeLa- and A204-established lines was similar to that with the diploid MRC-5 cells and with the human cells continuously producing RD-114 virus (Fig. 1). However, A204 cells chronically infected with BaEV(M7) appeared to have more binding sites for antibody D-12. This unexplained finding was repeatedly observed when A204(M7) cells were compared with other virus-free human cell lines (data not shown). Virus-coded proteins were not responsible for the increased binding of antibody D-12 since the bat cells CCL-88 and the canine Fcf2th cells did not bind the antibody, even when they were chronically producing M7 or BILN strains of BaEV, respectively.

Binding of IgM antibody E-4 was monitored with an intermediate treatment by rabbit anti-mouse IgM antibodies before ^{125}I-labeled protein A addition. The reaction patterns of antibody E-4 were similar to those shown with antibody D-12.

Competition between the two antibodies was studied previously by cytofluorometry with fluorescent anti-IgG2a and anti-IgM antibodies. Prior cell treatment with antibody E-4 at 4°C completely inhibited antibody D-12 adsorption, whereas prior treatment with antibody D-12 only partially interfered with antibody E-4 binding (6). In addition, we showed that prebound antibody D-12 was displaced after incubation with antibody E-4 at 37°C, whereas the reverse was not true (Table 2). HeLa cells treated with antibody D-12 for 1 h at 4 or 37°C became fluorescent after addition of anti-IgG2a fluorescein isothiocyanate conjugate, but the reaction was negative if antibody D-12 treatment was followed by incubation of the cell with antibody E-4 for 1 h at 37°C. Incubation at 4°C did not cause disappearance of antibody D-12, indicating that antigen-antibody E-4 complexes trigger an active process at 37°C which modifies a neighboring site with specificity for antibody D-12. By contrast, rabbit anti-mouse IgM serum reacted to the same extent with E-4-treated cells whether antibody D-12 was subsequently added or not.

The human antigen recognized by antibodies D-12 and E-4 is probably not situated within the HLA site. We treated Hep-2 cells, which express a high amount of HLA (6), with IgG2a antibodies Mas 1532, reacting against a determinant shared by HLA-A, -B, and -C, and Mas 018

TABLE 1. Comparative binding of the monoclonal antibodies to disrupted virions: radioimmunoassay with iodinated protein A

Antibody[a]	Amt (cpm of ^{125}I-labeled protein A bound) with the following viral antigens[b]		
	M7 (A204)	M7 (CCL-88)	RD-114
A-6	12,989 ± 468	8,019 ± 41	240 ± 2
C-9	5,237 ± 128	2,265 ± 49	224 ± 2
F-12	9,471 ± 902	5,466 ± 636	1,166 ± 101
D-12	11,360 ± 1,042	229 ± 92	382 ± 22
E-4[c]	5,903 ± 452	94 ± 10	347 ± 10
None	236 ± 6	93 ± 3	197 ± 36

[a] Culture supernatant diluted 1:16.

[b] 50,000 cpm added per well. Results are reported as the means ± standard deviations of counts per minute bound in four wells.

[c] The assay for IgM antibody E-4 was performed with an intermediate addition of rabbit anti-mouse IgM antibody before ^{125}I-labeled protein A treatment.

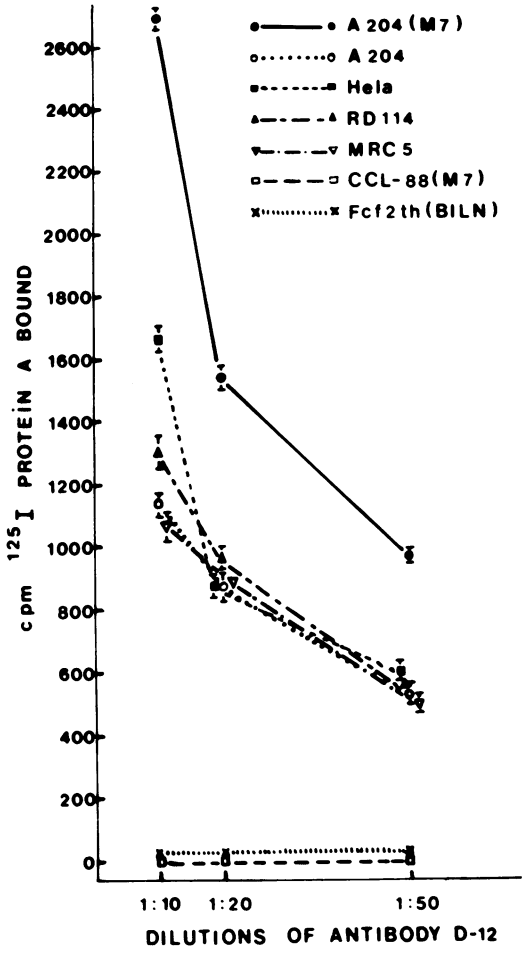

FIG. 1. Assay for antibody D-12 binding to various virus-free and virus-producing human and animal cells. Hybridoma culture medium with antibody (50 μl) was mixed at three dilutions with 5×10^4 cells for 1 h at 37°C and then for 1 h at 4°C. Washed cells were then treated with 4×10^4 cpm of ^{125}I-labeled protein A for 30 min at 37°C and bound counts per minute were counted. Cell lines tested and their symbols are listed in the figure.

directed against β_2 microglobulin. There was no competition between adsorption of these antibodies and that of antibody E-4, as tested by cytofluorometry (data not shown). Reactivity of antibodies D-12 and E-4 was further analyzed by the Western blot technique applied to polypeptides of M7 virions from A204 cells and to membrane polypeptides prepared from virus-free and virus-producing A204 cells and denatured in sodium dodecyl sulfate. The antigen determinant recognized by the two antibodies appeared to reside on an 18,000-dalton protein in the three different preparations (Fig. 2). The intensity of the bands indicated that the virus-producing cell membranes contained more of this antigen than the normal cells, a conclusion in accordance with the results of radioimmunoassay shown in Fig. 1. It appeared also that the virus contained a higher proportion of the antigen than the cell membranes since only 50 μg of viral proteins were separated on the gel versus 350 μg of cellular proteins. One or two minor bands (16,000 and 13,000 daltons) were also detected in the virion polypeptides and may be due to partial degradation of the major protein. Antibody F-12, used as control, revealed a single antigen of 18,000 daltons in the viral preparation (Fig. 2A, lane 3), corresponding to the structural viral polypeptide already demonstrated by immunoprecipitation (5). This polypeptide was absent from the host cell membrane preparation (Fig. 2A, lanes 1 and 2). A similar analysis applied to bat cells, and M7 virus produced by these cells did not reveal any antigen corresponding to antibodies D-12 and E-4. In the same experiment, however, antibody C-9 used as a control reacted with a band at 85,000 daltons, as expected for the viral envelope gp in preparations from the virions and from virus-producing cells but not in those from virus-free cells (data not shown).

Inhibition of BaEV adsorption on host cells by antibodies E-4 and D-12. Indications that antibodies E-4 and D-12 may be directed at host receptors were previously obtained by showing that RSa cells pretreated with either of the two monoclonal antibodies and then washed were unable to form syncytia when challenged with BaEV, although syncytium formation by MP-MV was unaffected (5). We have now found that these two monoclonal antibodies do not inhibit the syncytia formed by RD-114 virus on the human RSa and KC cells (data not shown).

Retrovirus adsorption on other cell types was tested by mixing about 200 syncytium-forming units of BaEV, RD-114 virus, or MP-MV with 10^7 cells in suspension. After 1 h at 37°C, cells were spun and nonadsorbed units remaining in

TABLE 2. Detectable HeLa cell bound antibodies after treatment with a second antibody, as measured by quantitative flow cytofluorometry

First monoclonal antibody (undiluted)	Second monoclonal antibody (undiluted)	FITC[a] conjugate (1:20 dilution)	$\phi - \phi_A/\phi_F - \phi_A$[b]
None	None	Anti-IgG2a	1.0
D-12	None	Anti-IgG2a	4.0
D-12	E-4	Anti-IgG2a	0.6
None	None	Anti-IgM	1.0
E-4	None	Anti-IgM	11.1
E-4	D-12	Anti-IgM	10.4

[a] FITC, Fluorescein isothiocyanate.

[b] $\phi - \phi_A/\phi_F - \phi_A$ represents the ratio of mean fluorescence intensity per monoclonal antibody treated cell to that of control cell (see text).

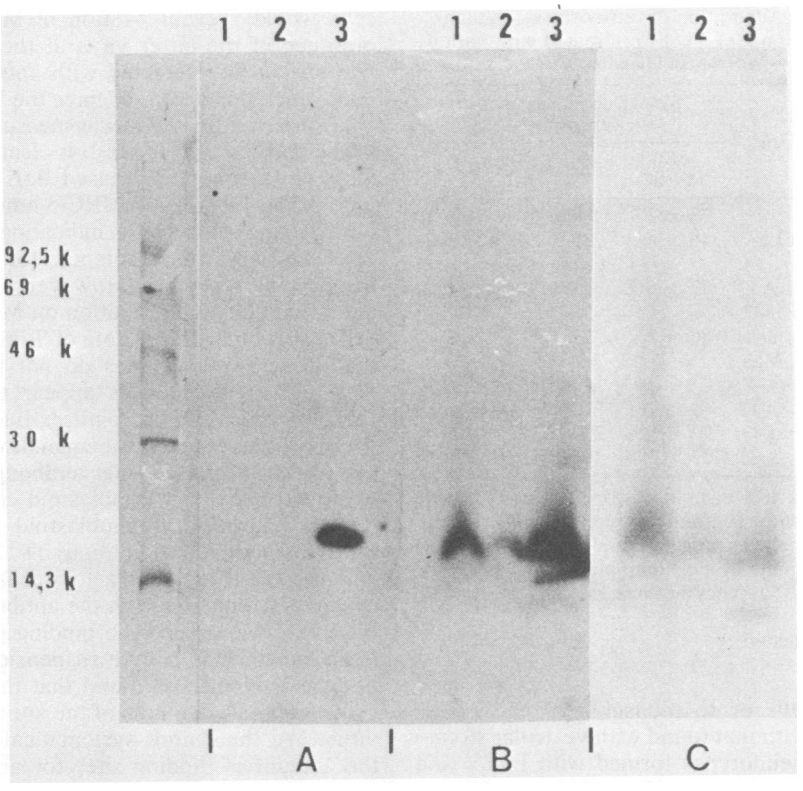

FIG. 2. Autoradiograph of the nitrocellulose blot from a 13% acrylamide gel containing (lane 1) A204(M7) membrane proteins, (lane 2) A204 membrane proteins, and (lane 3) M7 virus proteins. Each set of three antigens was incubated with (A) antibody F-12, (B) antibody D-12, and (C) antibody E-4, followed by rabbit anti-mouse IgM serum. Antibody binding was revealed by iodinated protein A. See text for further discussion of techniques.

the supernatants were counted by the syncytium-forming assay on RSa cells. To study inhibition of virus adsorption, cells were pretreated for 1 h at 37°C with various antisera and then washed before addition of the virus. An example of results obtained with HeLa cells is shown (Table 3). Heated heterologous rabbit antisera raised against HeLa cells inhibited adsorption of the three viruses on HeLa cells, whereas anti-HLA antibody Mas 1532 and anti-β_2 microglobulin antibody Mas 018 did not decrease adsorption of BaEV or MP-MV (RD-114 virus not tested with these antibodies). Antibodies D-12 and E-4 specifically blocked BaEV adsorption, with no effect on the fixation of the two other viruses.

Adsorption of BaEV onto susceptible cells was also measured by taking advantage of the fact that virus-free cells coated from without with BaEV bind IgG2a antibody A-6, which is directed at the gp of the virion. The cell-virus-antibody complexes will then bind ^{125}I-labeled protein A. In addition, blocking of virus adsorption by anticellular antibody can be assayed by pretreating the cells with antibody E-4 before addition of the virus. Since this antibody is of the IgM class, it will not be recognized by protein A. This test confirmed that antibody E-4 prevented adsorption of both BILN and M7 viruses onto the human A204 cells but did not inhibit BaEV adsorption on the simian Vero cells or on the canine Fcf2th cells (Table 4).

Table 5 summarizes the cell adsorption patterns of RD-114 virus, MP-MV, and BaEV and the effect of antibodies D-12 and E-4 on virus fixation. Adsorption of the three viruses was measured by titrating nonadsorbed syncytium-forming units after cell contact. In addition, adsorption of BaEV was also evaluated with anti-gp monoclonal antibody A-6. This test does not apply to MP-MV and RD-114 virus whose gps are not recognized by antibody A-6. Among the cells tested, only human and simian cells adsorbed MP-MV, whereas receptors for BaEV were broadly distributed among human and animal cells, with the exception of NC37 cells which adsorbed MP-MV but fixed neither BaEV nor RD-114 virus. However, host range for adsorption of these last two viruses was not identical since RD-114 virus did not bind to the

TABLE 3. Adsorption of retrovirus syncytium-forming units on HeLa cells pretreated with various antibodies

Cell pretreatment	Syncytium-forming units in supernatant[a]		
	MP-MV(B7)	BaEV(M7)	RD-114
Medium	78 ± 7[b]	68 ± 8	64 ± 10
Rabbit anti-HeLa cell serum	216 ± 4	239 ± 22	123 ± 20
Mouse anti-HLA IgG2a, Mas 1532	77 ± 6	65 ± 7	NT[c]
Mouse anti-β_2 microglobulin, Mas 018	80 ± 5	73 ± 6	NT
Mouse IgG2a, D-12	69 ± 7	212 ± 17	49 ± 16
Mouse IgM, E-4	72 ± 6	220 ± 19	44 ± 4
No cells	223 ± 14	202 ± 12	149 ± 52

[a] Virus was left in contact for 1 h at 37°C with medium or with 10^7 antibody-treated HeLa cells, followed by cell centrifugation and titration of syncytium-forming units in the supernatant.

[b] Mean of RSa syncytia in eight wells ± standard deviation.

[c] NT, Not tested.

rat NRK cells or to mouse L929 cells. This pattern fits with that found with vesicular stomatitis virus pseudotypes formed with BaEV and with RD-114 virus (16). We found, in addition, that T lymphoblasts of the Molt cell line also permitted BaEV to be differentiated from RD-114 virus, since only the former virus adsorbed on these cells.

Even in the human cells susceptible to both BaEV and RD-114 virus, the virus receptors were not identical since only BaEV adsorption was prevented by antibodies D-12 and E-4. These results were surprising since reciprocal interference between the two viruses has been demonstrated; prior infection of susceptible cells with the M7 strain of BaEV or with RD-114 virus interfered with their transformation by MSV(M7) and MSV(RD-114) pseudotypes (8). In view of this contradiction, we tested whether prior adsorption of RD-114 virions on various cells would prevent fixation of M7 virus. The presence of the latter virus at the cell surface was specifically detected with anti-gp antibody A-6, which does not recognize the RD-114 virus gp. Bound antibody A-6 was measured with ^{125}I-labeled protein A (Fig. 3). It is clear that RD-114 virus pretreatment decreased BaEV adsorption onto A204, Hep-2, and MRC-5 human cells and onto bat and canine cells, indicating that in these cells the two viruses compete for the same or neighboring receptors. However, RD-114 virus did not inhibit BaEV fixation on Molt and L929 cells. This fits with the data of Table 4, showing that these two cell lines do not bind RD-114 virus. Thus, these cells appear to possess a receptor which only recognizes BaEV.

Correlation between the amount of cell receptors for BaEV and those for antibody D-12. After we found that B lymphoblastoid cells from the Raji cell line and T lymphoblastoid cells from the Molt line bound both antibody D-12 and BaEV, whereas the B lymphoblastoid cells from NC37 cells reacted neither with the antibody nor with the virus, we studied the binding properties of fresh human lymphocyte suspensions from the peripheral blood and found that these lymphocytes fixed low amounts of the antibody and the virus. We then more systematically compared the amount of binding sites for antibody D-12 and BaEV in various human cell suspensions. One sample of cell suspension was treated with antibody D-12, followed by ^{125}I-labeled protein A, and another sample was treated first with BILN virions and then with antiviral antibody A-6 and ^{125}I-labeled protein A. There was a clear correlation between the amount of cell sites for antibody D-12 and the receptors for BaEV (Fig. 4).

Lymphocytes from the peripheral blood of three adults fixed a significant but small amount of ^{125}I-labeled protein A (in counts per minute) after treatment with antibody D-12 or BaEV plus antibody A-6, and similar results were achieved with cell suspensions obtained after trypsinization of liver, kidney, and lung fragments of a 12-week-old human fetus. The kidney cells were

TABLE 4. Inhibition of BaEV adsorption by cell pretreatment with antibody E-4[a]

Cell treatment[b]	Amt (cpm of ^{125}I-labeled protein A) adsorbed with the following virus and cell combinations:			
	BILN	M7		
	A204	A204	Vero	Fcf2th
Virus-antibody A-6	290 ± 13[c]	286 ± 11	305 ± 33	402 ± 34
Antibody E-4-virus-antibody A-6	12 ± 4	13 ± 5	298 ± 27	420 ± 40
Antibody E-4	19 ± 6	16 ± 4	19 ± 3	31 ± 5
None	17 ± 5	26 ± 4	15 ± 4	27 ± 2

[a] Adsorbed virus was evaluated with anti-gp antibody A-6 plus ^{125}I-labeled protein A.

[b] Sequential treatments for 1 h at 4°C.

[c] Mean ± standard deviation for tests run in quadruplicate.

TABLE 5. Host range for adsorption of three retroviruses and inhibition of the adsorption by antibodies E-4 and D-12[a]

Cells	Virus adsorption			Inhibition by antibody		
	MP-MV[b]	RD-114[b]	BaEV[b,c]	MP-MV[b]	RD-114[b]	BaEV[b,c]
Human						
A204	+	NT	+		No	Yes[e]
RSa	+	+	+	No	No	Yes
HeLa	+	+	+	No	No	Yes
Hep-2	+	+	+	No	No	Yes
NC37	+	−	−			
Molt	NT[d]	−	+			Yes
Raji	NT	NT	+			Yes
Simian Vero	+	+	+	No	NT	No
Bat CCL-88	−	+	+			No
Canine Fcf2th	−	NT	+			No
Rat NRK	NT	−	+			No
Mouse L929	NT	−	+			No

[a] Summary of data obtained with two methods.
[b] Virus adsorption on cell suspensions and titration of syncytium-forming units in the cell supernatant, as described in Table 3.
[c] BaEV adsorption monitored by treating the coated cells with antiviral antibody A-6, as described in Table 4.
[d] NT, Not tested.
[e] Significant inhibition (Yes) of virus adsorption by antibodies E-4 and D-12 was calculated by the Student *t* test from data similar to those shown in Tables 3 and 4.

cultivated for 2 weeks, and cells of this primary culture showed an increased number of binding sites for antibody D-12 and BaEV. However, except for NC37 cells, the number of binding sites was much greater in the established cell lines. Cells suspended by trypsin treatment showed even more binding sites than those suspended with glass beads. Syncytiotrophoblastic cells from three term placentas had more binding sites per cell than any other cells freshly obtained from human tissues.

Influence of BaEV adsorption on subsequent binding of antibody D-12. After showing that antibody D-12 prevented BaEV adsorption, we investigated whether cells with adsorbed virus were still able to bind the antibody. Virus pellets prepared from the culture medium of various virus-producing cell lines were added to monolayers of HeLa or Fcf2th virus-free cells and left for 2 h at 4°C before washing. Cells were suspended in PBS-glucose and samples were sequentially treated with various dilutions of antibody D-12 and then ^{125}I-labeled protein A. When HeLa cells had been pretreated with BaEV(BILN) grown in Fcf2th cells, their ability to bind antibody D-12 was decreased as compared with HeLa cells not treated with the virus (Fig. 5A), and a similar result was obtained if the cells were treated with BaEV(M7) grown in CCL-88 cells (data not shown). By contrast, cell pretreatment with RD-114 virus did not modify antibody D-12 binding (Fig. 5B).

Surprisingly, when the virus-free canine Fcf2th cells were treated with BaEV grown on

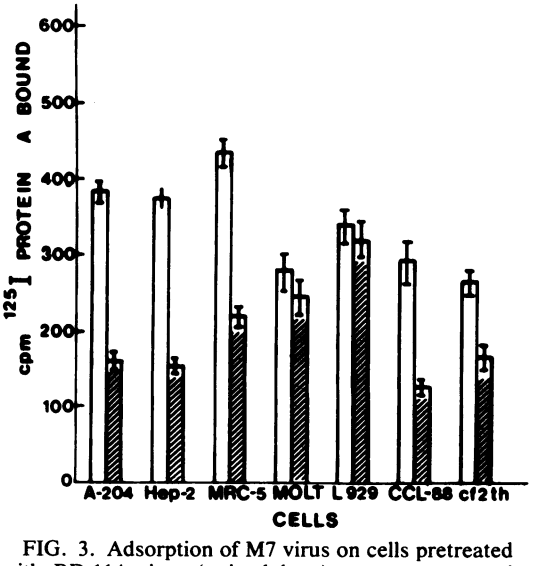

FIG. 3. Adsorption of M7 virus on cells pretreated with RD-114 virus (striped bars) or not pretreated (open bars). After contact with the viruses, the cells were treated with antiviral antibody A-6 and then with ^{125}I-labeled protein A, as described in the text.

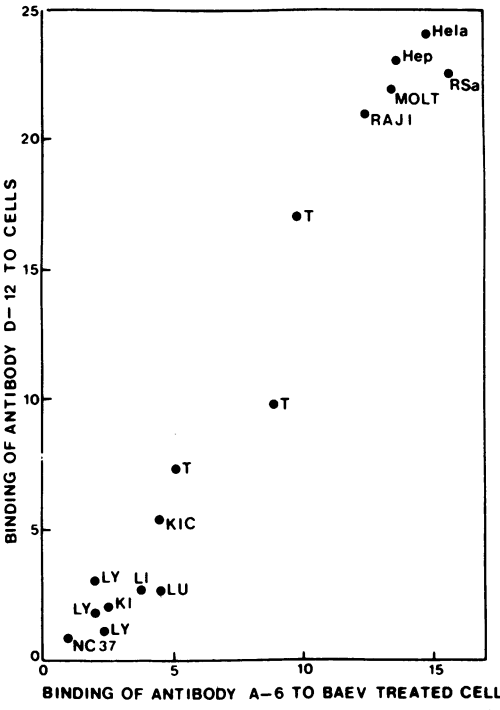

FIG. 4. Comparison of the amount of cell binding sites for antibody D-12 (ordinate) to binding sites for BaEV, as detected by antiviral antibody A-6 (abscissa). Various suspensions of 8×10^5 cells were divided into two samples, one of which was treated with 10^5 syncytium-forming units of BILN virions, washed, and distributed into four wells and then treated with antibody A-6 diluted 1:5. The other sample was directly distributed into wells without virus pretreatment and was assayed for binding of antibody D-12 diluted 1:5. Binding of the two antibodies was assayed with ^{125}I-labeled protein A. Indices of fixation of this protein were calculated by dividing the amount of counts per minute bound onto cells plus antibody by the amount bound to cells alone. Suspensions of noncultivated cells were lymphocytes of the peripheral blood (LY); syncytiotrophoblastic cells from three placentas (T); liver, lung, and kidney cells from a 12-week-old fetus (Li, Lu, and Ki, respectively). A primary culture of this kidney suspension (KIC) was also studied, as well as cells from six lines designated in the graph.

A204 cells, they acquired the ability to bind antibody D-12, although this antibody does not recognize animal cells. (Fig. 5C). This indicated that the human host cell antigen may be linked to the virus particles since it was carried over to animal cells by the virions. When BaEV of the human A204(M7) cells was adsorbed onto human cells, irregular and complex results were recorded, probably because on the one hand BaEV blocked some receptor sites for the antibody but on the other hand the adsorbed particles brought with them some human cell antigens which tend to react with antibody D-12.

Action of antibody E-4 on productive BaEV infection. When BaEV was added to A204 cells without prior polybrene treatment, the virus was detected at the cell surface with antiviral antibody A-6 for as long as 48 h, with eventual disappearance at 72 h. If cells had been pretreated with polybrene, most of the viral gp had disappeared from the cell surface at 6 h after virus addition, probably because of virus penetration. A latent period was observed at 12 and 24 h after infection, and viral gp was again detected at 72 h, most probably on budding progeny. When A204 cells were treated simultaneously with antibody E-4 and with the virus, no virus was adsorbed onto cells without polybrene, and no infectious cycle occurred after polybrene treatment (data not shown). We then studied the action of antibody E-4 on intracellular virus production in chronically infected A204(M7) cells as well as on the release of virus particles and of soluble viral gp. In cells grown with antibody E-4 for 48 h, there was an accumulation of the internal viral polypeptide recognized by antibody F-12, and there was a slight increase of the viral gp detected by antibody A-6 (Fig. 6A). Viral protein accumulation probably resulted from inhibition of release, since medium from the antibody-treated cultures contained fewer virus particles in the ultracentrifugation pellet (Fig. 6B) and less viral soluble gp in the supernatant (Fig. 6C). In contrast to antibody E-4, anti-HLA antibody Mas 1532 did not modify the amount of virions and soluble gp released in the culture medium (data not shown).

We wanted to test whether production of RD-114 virus was also modified by antibody E-4. It was not possible to monitor the synthesis of RD-114 gp because of the lack of appropriate antibodies. However, antibody F-12 recognizes an internal polypeptide which is common to BaEV and RD-114 virus. When the RD-114 cells were cultivated for 48 h in the presence of antibody E-4, there was no modification of the amount of intracellular viral protein corresponding to antibody F-12, indicating that virus release was not inhibited (data not shown).

DISCUSSION

We have found that antibodies D-12 and E-4 defined two epitopes lying within an 18,000-dalton protein present in the membrane of human cells but not in that of other animal species. The two determinants either possess partial overlapping sequences or are distinct but topographically close to each other. Cell treatment with IgM antibody E-4 at 4°C or 37°C completely precluded the binding of IgG2a antibody D-12. If cells were treated first at 4°C with antibody D-12 and then with antibody E-4, both antibodies could be detected by cytofluorometry. Howev-

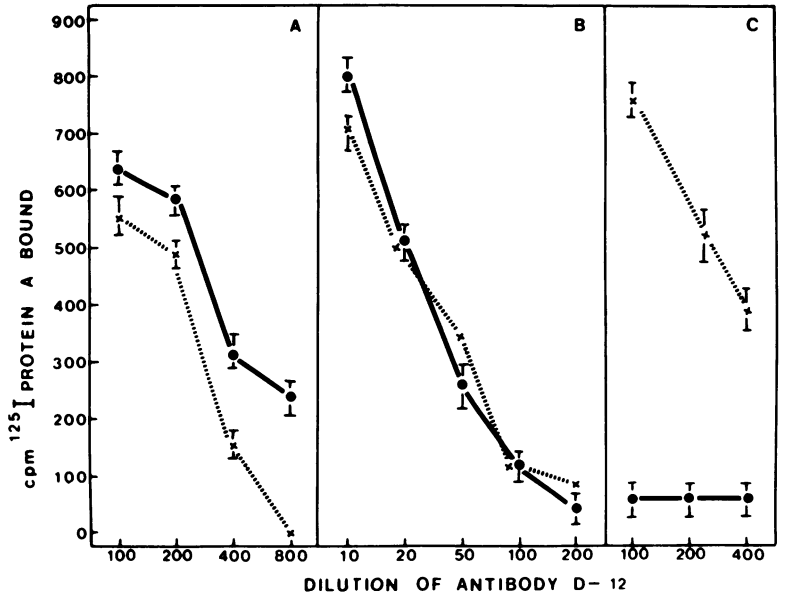

FIG. 5. Binding of antibody D-12 on cells with adsorbed BaEV or RD-114 (×) or on virus-free cells (●). Supernatants of 3- to 4-day-old cultures of virus-producing cell lines, containing ca. 1,000 syncytium-forming units per ml, were ultracentrifuged to achieve a 50-fold concentration of the virus. Virus suspension (30 ml) was added to monolayers of 5×10^6 virus-free cells for 2 h at 4°C. After washing, cells were suspended in PBS-glucose solution and distributed into wells of microplates in which serial dilutions of antibody D-12 were added, followed by addition of ^{125}I-labeled protein A, as described in the text. For adsorption, combinations of viruses plus cells were as follows: (A) BaEV(BILN) grown in Fcf2th cells adsorbed on HeLa cells; (B) RD-114 virus grown in RD-114 cells adsorbed on HeLa cells; and (C) BaEV(M7) grown in A204 cells adsorbed on Fcf2th cells.

er, further incubation of these cells at 37°C caused the disappearance of antibody D-12 only. One may assume that the formation of the antigen-antibody E-4 complex caused, at 37°C, capping phenomena or allosteric modifications of the sites reacting with antibody D-12, resulting in release or endocytosis of this antibody.

Although these antibodies do not recognize identical sites, we could not disclose differences in their cellular effect in experiments in which the action of both antibodies was compared. Cell binding was more readily assayed with IgG2a antibody D-12 plus ^{125}I-labeled protein A. Conversely, it was convenient to study the effect of IgM antibody E-4 on viral infection by subsequent detection of the appearance of viral proteins with the IgG2a antiviral antibodies plus iodinated protein A. Binding of antibodies D-12 and E-4 onto cells from different human lines was sufficient to inhibit cell adsorption of BaEV syncytium-forming units. These units were measured by counting the number of syncytia produced in RSa cells. With BaEV, syncytia may be caused either by the virions or by soluble viral gps which are released in excess of virus particles into the culture medium of BaEV-infected cells. Soluble gp from supernatants of the ultracentrifuged medium did not produce syncytia on RSa cells if these cells had been pretreated with antibodies E-4 or D-12, indicating that the receptors masked by the antibodies were actually the binding sites for the viral gp.

We also set up another assay for BaEV adsorption by demonstrating that target cells coated with this virus bound antibodies C-9 and A-6 which are directed at the viral gp70. With this assay, we confirmed that prior treatment of human cells with antibody E-4 precluded BaEV adsorption. Evidence that virions and antibody D-12 competed for the same cellular site also derived from the observation that cells coated at 4°C with BaEV from dog or bat cells bound less antibody D-12 than virus-free cells.

Another indication that target sites for antibodies E-4 and D-12 are receptors for BaEV was obtained when antibody adsorption on various human cells was quantitated and was found to correlate with the number of BaEV particles that each cell type was able to adsorb. Cell suspensions directly prepared from human blood or fetal tissues possessed very low amounts of BaEV receptors, and this probably reflects the in vivo situation. Placental syncytiotrophoblastic cells bound more antibody D-12 than the other tissues tested, but this does not necessarily prove that receptor density was greater, since

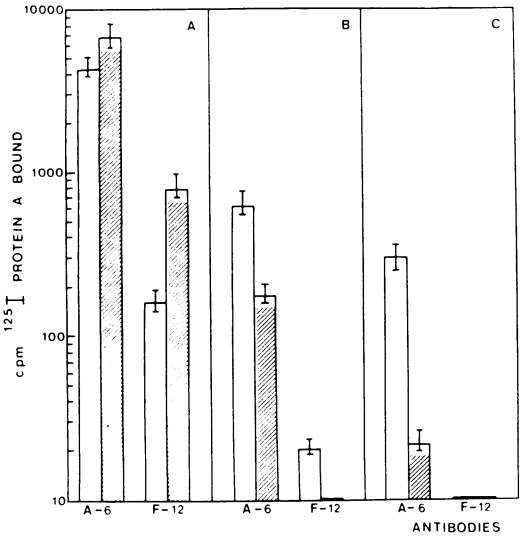

FIG. 6. Influence of antibody E-4 on virus production by chronically infected A204(M7) cells. The cells were grown for 48 h with antibody E-4 1:10 (striped bars) or without it (open bars). Medium was then collected and ultracentrifuged, and the cells were frozen at −80°C and then sonicated. The presence of (A) BaEV proteins was estimated in broken cells, (B) virus particles in the pellet of the culture medium, and (C) soluble gp in the supernatant of the centrifuged culture medium. BaEV gp was measured with antibody A-6 plus ^{125}I-labeled protein A, and an internal viral polypeptide was detected with antibody F-12 plus ^{125}I-labeled protein A.

the amount of antibody binding was counted for a given number of cells and the syncytia of the trophoblast are giant cells. Induction of BaEV receptor expression may be related to the cell proliferative state or may be derepressed in vitro, since antibody D-12 binding was high in human cells from various established lines. Induction of receptor expression is not a rapid in vitro event, since no clear-cut increase of cell receptors occurred within a 4-day culture of lymphocytes with 5 μg of phytohemagglutinin or other mitogens per ml. In contrast, one human T lymphoblastoid cell line (Molt) and one B lymphoblastoid cell line (Raji) expressed a high number of binding sites for BaEV. It is unclear why NC37 cells bound neither the virus nor antibodies E-4 and D-12.

Our data indicate that the determinants corresponding to antibodies D-12 and E-4 are, on the one hand, situated close to or within the BaEV receptor and, on the other hand, within an 18,000-dalton protein. This amount is not very different from the value of 14,000 daltons found for the Friend murine leukemia virus receptor on mouse spleen leukocytes (13) nor from the 10,000-dalton protein isolated from the spent medium of BALB/c 3T3 cells which specifically bound gp70 of Rauscher murine leukemia virus (10). However, it differs from the complex of two 190,000-dalton units found on the murine lymphoid cell surface (14), but the authors considered the possibility that a small molecule might be associated with this heavy complex, although their experimental conditions did not allow its demonstration on the gel used. Retroviruses may associate with histocompatibility antigen (4), but the human-specific epitopes recognized by antibodies D-12 and E-4 were not linked to the heavy chain of HLA or to β_2 microglobulin proteins which have molecular weights of 45,000 and 11,000, respectively. Since the D-12 and E-4 antibody sites were absent of BaEV-permissive canine and bat cells, the viral gps may possess other determinants which enable them to attach to animal cells. As an alternative, BaEV receptors from human and animal species may possess the same active site, but in human cells this site may be flanked by an adjacent epitope(s) reacting with antibodies D-12 and E-4.

As expected, cell treatment with antibody E-4 not only interfered with virus binding but, as a consequence, also inhibited appearance of new viral progeny when A204 cells were exogenously infected with BaEV after antibody treatment. It was more surprising to observe that antibody E-4 applied to A204 cultures chronically infected with BaEV caused intracellular accumulation of an internal viral polypeptide recognized by antibody F-12 and decreased the release of free virions and soluble gp in the culture medium. One would expect an accumulation of extracellular virus if antibody E-4 only inhibited readsorption of newly synthesized viral progeny. The results indicated that interaction of antibody with the cell receptor may interfere with virus maturation or release or both and thus suggested that the receptor may also play a part at the final stage of the virus infectious cycle.

At this stage, some specific association between the virions and determinants of the cell receptor also seems to occur. The assumption is based on the following evidence. Antibodies E-4 and D-12 were obtained from mice immunized with purified BaEV which had been grown in the human A204 cells. These antibodies were first detected during a screening which aimed at selecting antiviral monoclonal antibodies and used purified BaEV as antigens. Clearly, these virus preparations contained cell antigens since they bound antibodies E-4 and D-12, which were later found to react with virus-free cells. However, cell antigens of the virus preparations were probably not randomly or loosely associated with the virions. RD-114 virus grown in human cells did not contain these cell determinants.

The human cell determinants of BaEV coadsorbed with the virions onto animal cells, showing that BaEV could play the part of a vehicle to carry a human antigen over to an animal cell. This human antigen even appeared to be concentrated on the virions, since BaEV particles reacted more strongly with antibodies D-12 and E-4 than did cell membrane preparations of virus-free cells. Also, A204(M7) cells chronically producing BaEV reacted more strongly with antibody D-12 than did the control virus-free cells. This contrasts with feline leukemia viruses grown in human cells, which picked up HLA antigens but did not selectively concentrate HLA determinants either in the host cell or in the virions (1).

If the cell receptors were really complexed with some spikes of the viral envelope, other gp70 units must remain free for virus fixation and infection to occur, and stripping of the receptors from the particles could lead to increased chances for infectious processes to occur. On the other hand, if each cell receptor was able to bind more than one viral gp70, a dense suspension of virus particles would tend to agglutinate and produce networks with receptor bridges because links could occur between one particle with a gp70 receptor complex and another virus particle with a free gp70. Another possibility is that the cell epitopes described above might become integrated within the viral envelope during maturation, independently of the gp70.

The use of monoclonal antibodies helped to further characterize the differences between BaEV and the closely related RD-114 virus as well as between their cell receptors. Two determinants of BaEV gp70 have been characterized by their reaction with antibodies A-6 and C-9 on the one hand and with antibody B-9 on the other hand (6), and we showed here that they were not recognized on RD-114 virus. However, gps of the two virions probably address overlapping or adjacent receptors, since human amnion cells infected with RD-114 virus or with BaEV were resistant to focus formation by pseudotypes of Moloney sarcoma virus with BaEV or RD114 virus envelopes, respectively (8).

We also showed that prior adsorption of RD-114 virus on human cells decreased the cells ability of the cells to bind BaEV. However, host ranges for adsorption of the two viruses bear some differences. It had already been demonstrated that vesicular stomatitis virus pseudotypes with BaEV envelopes adsorbed to mouse and rat cells, whereas the pseudotypes with RD-114 virus did not (16). We confirmed that NRK and L929 cells adsorbed syncytium-forming units of BaEV but not those of RD-114 virus, and we also found that human Molt cells bound BaEV only. In addition, determinants recognized by antibodies E-4 and D-12 are not within the active site of the RD-114 virus receptors, since these antibodies did not block adsorption of the virus and since, conversely, prior adsorption of the virus onto human cells did not decrease antibody D-12 binding. It was interesting to note that when RD-114 cells chronically producing the virus were grown for 48 h in the presence of antibody E-4, there was no intracellular accumulation of the viral polypeptide recognized by antibody F-12, in contrast to the data with BaEV. Also, there was no association of RD-114 virions with the human determinants recognized by E-4 and D-12, since purified viruses did not bind these antibodies in a solid-phase radioimmunoassay, and since these human determinants did not coadsorb with RD-114 virus on the membranes of canine cells. Thus, the effects of antibodies D-12 and E-4 on virus adsorption, virus release and virion association with the human cell determinants could not be dissociated from each other and were virus specific.

The presence of receptors on the cell membrane is necessary, but it is not the only condition for productive viral infection. For instance, we showed that mouse cells adsorb BaEV although these cells are not virus permissive, probably because they lack an integration site in their chromosome (12). This site is present in the human chromosome 6 (11), and another cell function necessary for BaEV infection is coded by chromosome 19 (3). Such a function may relate to the synthesis of polypeptides common to BaEV and RD-114 virus receptors, since the receptor for the latter virus is coded by human chromosome 19 (15). It will be of interest to study whether the BaEV-specific polypeptide sequences recognized by antibodies D-12 and E-4 are also coded by the same chromosome. It will also be possible to directly test whether the presence of chromosome 19 is necessary for BaEV adsorption, since cells with acceptor sites for the virus will subsequently bind antiviral antibodies A-6 or C-9.

ACKNOWLEDGMENTS

This work was supported by grants from Fonds de la Recherche Scientifique Médicale and Fondation Hoguet. R.O. and P.B. are "Aspirants du Fonds National de la Recherche Scientifique."

We thank D. L. Fine, M. Ahmed, and P. Jacquemin for providing the retrovirus-producing cells. The excellent technical assistance of F. Berdal, M. L. Blondiau, M. Jacques, J. Stienon, and L. Tack was greatly appreciated. We are grateful to J. Herinckx for preparing the manuscript.

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