JOURNAL OF VIROLOGY, Aug. 1982, p. 664–672
0022-538X/82/080664-09$02.00/0

Vol. 43, No. 2

Monoclonal Antibodies Against Baboon Endogenous Virus and Against Host Cell Antigens

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Received 18 December 1981/Accepted 6 April 1982

Monoclonal antibodies were produced by murine hybridomas after immunization with semipurified baboon endogenous virus. In a solid-phase radioimmunoassay, two antibodies (F12-9 and B9-18) reacted with viral antigen only. The antibodies A6-8 and C9-12 also reacted with virus-producing cells but not with control cells, whereas antibodies E4-6 and D12-2 bound to virus-free cells as well. The cytofluorometry technique confirmed these results and showed a competition between antibodies A6-8 and C9-12 for binding to virus-producing cells as well as a competition between antibodies D12-2 and E4-6 for binding to virus-free human cells. An immune precipitation assay with disrupted virions indicated that antibodies A6-8, B9-18, and C9-12 were directed against the gp70 glycoprotein, and that antibody F12-9 reacted with a viral antigen with a molecular weight of 18,000. The syncytia induced in RSa cells by baboon endogenous virus could be inhibited either when antibody A6-8 or C9-12 was combined to the virus or when the RSa cells were treated with the anticellular antibody D12-2 or E4-6. These two effects were not observed with Mason-Pfizer virus. Thus, of three antibodies with specificities for viral gp70, two (A6-8 and C9-12) were directed at viral sites responsible for syncytium formation. Another antiviral antibody (F12-9) reacted with a protein of unknown function with a molecular weight of 18,000. The two anticellular antibodies were directed at similar or neighboring epitopes, which may be situated within the receptor to the virus.

Monoclonal antibodies have been produced against proteins from several types of viruses, including retroviruses. Hybridomas for retroviruses have been obtained by fusion of mouse myeloma cells with spleen cells from mice immunized with allogeneic virus-producing cells (17, 20) or purified virus (7, 18). Some of these very specific antibodies have provided insight into the topographical arrangement of the proteins which constitute the envelope of murine leukemia virus (MuLV) (22). Further investigation has revealed the arrangement of nine distinct epitopes on the two virion envelope proteins, one of them being responsible for ecotropism. The existence of functional sites related to structural domains in the gp70 glycoprotein of MuLV (21) could thus be demonstrated. Multiple epitopes also have been defined on the proteins of mouse mammary tumor virus with a panel of monoclonal antibodies, and the individual polypeptides of the virus have been found to be expressed differently in various murine mammary tumors (7).

By comparing different strains of mouse mammary tumor virus (18) or MuLV (6) in terms of their reactivity with distinct, specific monoclonal antibodies directed against the envelope glycoproteins, it appeared for the first time that the patterns of class, group, and type specificity are related to distinct antigenic determinants.

We report here the identification and isolation of hybrid clones secreting antibodies against proteins of baboon endogenous virus (BaEV) or against host cell components. This xenotropic type C simian retrovirus has been isolated by Benveniste et al. (1). It multiplies preferentially in human or dog cells and has been shown to be present in a naturally integrated state within the primate genome (2). To our knowledge, no monoclonal antibodies to simian retroviruses have been previously described.

The specificities of the immunoglobulins which we obtained were assayed by studying binding to viral and nonviral antigens, by radioimmunoassay (RIA), or by cytofluorometry. Antiviral neutralizing activity was studied by taking advantage of the fact that BaEV produces syncytia on RSa cells (28).

MATERIALS AND METHODS

Viruses and cells. Mason-Pfizer monkey virus (M-PMV) was obtained from a high-virus-producing variant of infected monkey foreskin cell line (MPFS clone 940C3 or Rhfs-B7) kindly provided by D. L. Fine. BaEV of placental origin (strain M7) was produced by human A204 cells, whereas strain BILN from inguinal

lymph node (11) was cultivated in canine Fcf2th cells. The RSa human embryonic cell line transformed by Rous sarcoma virus and simian virus 40 (15) was used for detecting syncytium formation by BaEV and M-PMV. Cells were grown in Dulbecco modified Eagle medium with 10% fetal calf serum, except for A204 cells, which were cultivated in McCoy 5A medium. HeLa cells were maintained in spinner culture with Joklik modified Eagle medium with 10% fetal calf serum. The Sp2/0 derivative from the BALB/c P3-X63-Ag8 myeloma line isolated by Shulman et al. (25) was chosen to generate hybrid cells. It was grown in Iscove modified Dulbecco medium (powdered form; GIBCO Laboratories) supplemented shortly before use with 1% 200 mM glutamine–2% 100 mM sodium pyruvate–10% inactivated fetal calf serum. As recommended by Franssen et al. (10; personal communication), the Sp2/0 myeloma cells were recloned twice in soft agar in the presence of 25 mM 8-azaguanine. Some of the cells were transplanted into the peritoneal cavity of BALB/c mice and reisolated in vitro. The clones showing the highest growth rate were selected for fusion.

Virus concentration. BaEV strain M7 was concentrated from the culture medium of virus-producing A204-M7 cells so that it could be used as antigen for the immunization of mice and for the detection of antibodies by RIA. Medium from confluent cultures in roller bottles was harvested every 2 days and clarified at 5,800 × *g* for 20 min. Particles from the supernatant were concentrated 10 to 20 times by circulation through Diaflo hollow fibers (type HIX50; Amicon Corp.). The concentrate was centrifuged at 105,000 × *g* for 1 h in a 50.2 Ti rotor (Beckman Instruments, Inc.). The virus-containing pellet was suspended in phosphate-buffered saline (PBS) (pH 7.2) and again sedimented. The 1,000-fold concentrate of virus-containing culture medium was subjected to three cycles of freezing in liquid nitrogen followed by thawing or to sonication and kept frozen at −70°C. The suspension was clarified at 1,000 × *g* for 10 min before use as antigen. Protein concentration was determined by the method of Bradford (4).

Mouse immunizations. The two different schedules described by Massey et al. (18) were applied. Five-week-old BALB/c mice were inoculated subcutaneously with 75 μg of strain M7 viral antigen in complete Freund adjuvant. They were then boosted on days 7 and 14 by intraperitoneal inoculations of 100 μg of BaEV strain M7, and the spleens were removed 4 days later for fusion 1. Mice in the second group received an additional intraperitoneal inoculation on day 21 and were sacrificed 4 days later for fusion 2.

Generation and selection of hybrid cell lines. The preparation of lymphocytes from spleens of immunized mice and the fusion procedure described by Fazekas de St. Groth and Scheidegger (8) were carried out with minor modifications. Briefly, the two cell populations were mixed at a ratio of two splenocytes to one Sp2/0 cell. After treatment with polyethylene glycol (50% in saline, added with 5% dimethyl sulfoxide) and subsequent dilution with saline, the cells were centrifuged at 200 × *g* and suspended in a large volume of medium containing 10% fetal calf serum. They were incubated at 37°C for 2 h, with gentle mixing every 15 min. After centrifugation, the cell pellet was suspended in selective HAT medium (8). The suspension was distributed with a Pasteur pipette into the wells of Costar tissue culture clusters (Costar Co.) that were seeded 1 day before with 5×10^3 peritoneal macrophages (from BALB/c mice) in 50 μl of complete HAT medium. Each well received approximately 10^5 spleen cells.

After 10 days, hybrid growth was visible in 63 and 76% of the wells seeded with cells from the fusion 1 and fusion 2 experiments, respectively. Of these cells, 21 and 15%, respectively, were identified as secreting antibodies against BaEV antigen by assaying the medium in a solid-phase RIA. Some of these positive cultures were discarded because they also reacted with the surfaces of normal A204 cells.

Solid-phase RIA. Hybrid supernatant fluids were assayed for reactive antibodies against viral or cellular antigens by the solid-phase binding assay described by Nowinski et al. (20). Strain M7 viral antigen was adsorbed to individual wells of a polyvinyl chloride microtiter plate (no. 1-220-24; Dynatech Laboratories, Inc.) by incubation overnight at 37°C (2 μg of protein per well). The binding of antibodies to the surfaces of fixed virus-producing (A204-M7) or control (A204) cells was assayed to discriminate between anticellular and antiviral antibodies (26). The cells were thoroughly washed in PBS and distributed into polyvinyl chloride microtiter plates (5×10^4 cells per well in 50 μl). They were sedimented to the bottom of the wells and fixed by adding 50 μl of 1% glutaraldehyde without removing the supernatant. After 1 h at 4°C, the cells firmly stuck to the plastic, and the plates were washed repeatedly by immersion in PBS and then washed once in 0.1 M ethanolamine to neutralize traces of glutaraldehyde. The plates were saturated with 1% bovine serum albumin in PBS with 0.02% sodium azide and kept at 4°C. The RIA was performed as described previously (20), with intermediate incubation with a 1:500 dilution of rabbit anti-mouse immunoglobulin M (IgM) serum. The binding of ^{125}I-labeled protein A was detected by 36-h autoradiography on Kodak X-OMAT R film with enhancement by an X-ray intensifying screen (27). When a quantitative analysis was necessary, the individual wells were separated with scissors, and the radioactivity was counted in an autogamma scintillation spectrometer (Packard).

Isolation of hybridomas. The hybrid cell cultures recorded as positive in the antibody binding assay were partly harvested when at 70% confluency and plated in petri dishes, 9 cm in diameter, for cloning in soft agar (12). Peritoneal macrophages (2.5×10^5 per dish) were seeded the day before. The bottom layer was made of 10 ml of 0.6% purified agar (Difco Laboratories) in complete Iscove medium supplemented with hypoxanthine–thymidine–20% fetal calf serum. The hybrid cells (2×10^3 to 2×10^4 per plate) were mixed in 2 ml of the upper layer, which contained 0.3% agar in the same nutrient medium. After 10 to 12 days, well-developed colonies could be transferred in 200 μl of culture medium to individual cups of Costar trays. Twenty-four isolated colonies were picked from each petri plate, and after a few days, their culture fluids were assayed for antibody production. Positive cultures were expanded gradually into larger flasks.

Characterization of immunoglobulins. Labeling of the immunoglobulins secreted by the hybrid clones was easily performed by incorporation of [^{14}C]leucine (250 μCi/μmol, Amersham Corp.) at a concentration

of 50 μCi/ml in a leucine-free medium supplemented with 2% dialyzed fetal calf serum. After a 20-h incubation period, a 20-μl sample of the culture fluid, clarified at 9,000 × *g* in a Beckman Microfuge, was mixed with sodium dodecyl sulfate (SDS) and mercaptoethanol in 0.1 M Tris buffer (pH 6.8). After boiling for 1 min, the denatured and reduced immunoglobulin chains were analyzed by electrophoresis on slab SDS-polyacrylamide gels, as described by Laemmli (16).

The immunoglobulin classes of antibodies produced by hybridoma cultures were determined by gel diffusion in 2% agarose in culture fluid concentrated 5- to 10-fold by ammonium sulfate precipitation (50% saturation). Rabbit antisera specific for mouse immunoglobulin classes were obtained from Nordic Immunological Laboratories.

Indirect immunofluorescence. Cells grown in monolayers were collected at 50% confluence with 1 mM EDTA. They were washed twice in cold PBS containing 2% fetal calf serum and 10 mM NaN_3. This medium was used throughout the immunofluorescence study to prepare dilutions of the monoclonal antibodies and of the fluorescein isothiocyanate (FITC)-conjugated IgG. Cell pellets containing 0.5×10^6 cells were suspended in 100 μl of the appropriate dilution of monoclonal antibody and incubated for 30 min at 4°C with frequent gentle agitation. Cells were then washed twice and stained for 30 min at 4°C with 100 μl of a 1:20 dilution of FITC-conjugated rabbit IgG directed against mouse immunoglobulin (RAM/Ig/FITC; Nordic Immunological Laboratories) or FITC-conjugated goat IgG specific for a particular type of mouse immunoglobulin (GAM/IgG2a/FITC, GAM/IgG2b/FITC, GAM/IgM(Fc)/FITC; Nordic Immunological Laboratories). Before use, FITC-conjugated IgG was clarified at 9,000 × *g* in a Beckman Microfuge. Stained cells were finally washed twice and diluted to 0.5×10^6 cells per ml for subsequent analysis by flow cytofluorometry.

Flow cytofluorometry. The flow cytofluorometer used was a Cytofluorograf 50-H (Ortho Instruments) equipped with two lasers. The 0.8-mW helium-neon laser, with an emission wavelength of 632.8 nm, was used for measurements of axial light extinction, which correlated approximately with cell size. The 5-W argon-ion laser (model 95; Lexel Corp.) was used for fluorescence excitation. The excitation wavelength was 488 nm for FITC. In all experiments, the laser power was 500 mW. A special interference filter with a band pass from 515 to 545 nm was placed in front of the green-channel photomultiplier to select the green fluorescence of FITC. Cells were processed at 250 cells per s, and the intensity of fluorescence (pulse height) was recorded for each individual cell on the pulse-height analyzer. Data on fluorescence intensity were displayed as histograms and were recorded channel by channel on a model 43 Teletype for further analysis. Each histogram is based on the analysis of 10,000 cells.

In some experiments, data were analyzed quantitatively by calculating the mean fluorescence intensity per cell (ϕ), determined according to the following formula (3):

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

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_{auto}, representing the cellular autofluorescence, was routinely subtracted from each reading. The level of background fluorescence (ϕ_{FITC}) was determined by analyzing appropriate negative controls, such as cells labeled with the FITC conjugate alone or cells treated with a nonreacting monoclonal antibody (such as F12-2; see below), before analyzing the FITC-conjugated IgG anti-mouse immunoglobulin. As these two kinds of negative controls gave the same background fluorescence levels, cells labeled with the FITC conjugate alone were considered as convenient negative controls.

Virus neutralization. Flat-bottomed wells of microplates were inoculated with 200 μl of Dulbecco modified Eagle medium containing 7×10^4 RSa cells. Subconfluent monolayers of cells obtained after 24 h of growth at 37°C in a humidified incubator with a 5% CO_2 atmosphere were treated with 20 μg of Polybrene per ml for 1 h. The medium was then removed, and 200 μl of a mixture of freshly clarified supernatants from 2-day-old cell cultures producing retroviruses and antibodies was added to the eight wells. The mixture contained antibodies at final dilutions of 1:10 to 1:100 and 85 to 120 syncytium-forming units of BaEV or 30 to 55 syncytium-forming units of M-PMV. It was incubated for 1 h at 37°C before inoculation to the cell monolayers. The microplates were left in the incubator for 24 h, and the cells were then fixed with methanol and stained with May-Grünwald Giemsa stain. The microplates were screened under a microscope equipped with a Dynascope (Vision Engineering Ltd.). One syncytium contained at least five nuclei, but most often, with RSa cells, there were 10 to 25 nuclei. Readings were made for eight wells, and the mean number of syncytia and the standard deviation were calculated. The percentage of syncytium-forming units which escaped neutralization was calculated according to the following formula: [(syncytia caused by virus + serum mixture)/syncytia caused by virus] × 100. There were no syncytia in the control RSa cells not inoculated with virus.

Immune precipitation assay. [^{3}H]leucine-labeled BaEV strain M7 was purified by density gradient centrifugation and solubilized in PBS containing 0.5%

TABLE 1. Characterization of immunoglobulins secreted by cloned hybrid cells

Clone	Immunoglobulin class [a]	Apparent mol w[b]	
		Heavy chain	Light chain
A6-8	G2a	53,000	25,000
B12-9	M	73,000	25,000
C9-12	G2b	56,000 (58,000)	25,000
D12-2	G2a	58,000	25,000
E4-6	M	74,000	23,500
F12-9	G2b	56,000 (58,000)	21,000
B9-18	G2b	56,000 (58,000)	21,000

[a] Determined by immunodiffusion.

[b] From SDS-polyacrylamide gel electrophoresis. Numbers in parentheses are the molecular weights of the minor heavy chains (when two sets of heavy chains were visible).

TABLE 2. Antibody binding assay in culture fluids from cloned hybrid cell lines

Clone	^{125}I-labeled protein A (cpm) bound on:		
	M7 viral antigen	A204-M7 cells[a]	A204 cells[a]
A6-8	27,168 ± 3,015	2,022 ± 353	421 ± 22
B12-9	2,808 ± 248	5,241 ± 222	4,712 ± 52
C9-12	24,888 ± 524	1,498 ± 48	226 ± 13
D12-2	10,525 ± 549	5,897 ± 722	4,996 ± 36
E4-6	1,240 ± 93	1,747 ± 68	1,026 ± 10
F12-2	11,806 ± 420	726 ± 15	281 ± 48
B9-18	2,829 ± 130	613 ± 99	605 ± 178
—[b]	461 ± 36	412 ± 15	375 ± 40

[a] Intact cells (10^5 per well) were fixed with glutaraldehyde as described in the text.
[b] —, Control culture fluid.

Nonidet P-40, 2×10^{-3} M phenylmethylsulfonyl fluoride, and 2×10^{-2} U of aprotinin per ml. The lysate was kept at 4°C for 1 h before centrifugation for 30 min at 100,000 × *g* to remove insoluble products. For immune precipitation reactions, 40 μl of virus extract (200,000 cpm) was added to 50 μl of undiluted or ammonium sulfate-concentrated culture fluid as an antibody source. The reaction mixture was incubated overnight at 4°C and then added to 50 μl of a 10% suspension of *Staphylococcus aureus* (13). The precipitate was collected by centrifugation (9,000 × *g* for 2 min, repeated twice) through a cushion of 10% sucrose in PBS containing 0.5% Nonidet P-40 and 0.5% deoxycholate and washed twice in the same buffer.

Immune complexes were eluted from *S. aureus* by incubation at 100°C for 2 min in 30 μl of electrophoresis sample buffer (1% SDS–5% 2-mercaptoethanol–10% glycerol in 0.06 M Tris-hydrochloride [pH 6.8]). Polyacrylamide gel electrophoresis was performed in 15% slab gels (18-cm long) according to Laemmli (16). After electrophoresis at 55 mA and 4°C for 3.5 h, the gels were treated for 30 min in salicylate (5) and fluorographed at −70°C.

RESULTS

The antibodies produced by seven hybrid clones were characterized by their isotypes and by their heavy- and light-chain components (Table 1). Electrophoresis of the labeled immunoglobulins under reducing conditions indicated a high homogeneity of molecular weight, except in the case of IgG2b, for which two bands were visible in the heavy-chain region, as reported previously by Köhler et al. (14).

The quantitative solid-phase RIA results reported in Table 2 show that the clones A6-8, C9-12, B9-18, and F12-9 produced immunoglobulins which preferentially bound to M7 viral antigen. B12-9 and E4-6 antibodies were bound in greater amounts to the cells, whether virus producing or not. It was presumed that they were directed against cellular antigens. We speculate that the high value obtained for the D12-2 antibody against the viral antigen was due to its binding to cellular antigen concentrated into the BaEV strain M7 preparation. The specificity of these seven monoclonal antibodies has been studied further, as described below.

Indirect immunofluorescence and flow cytofluorometry. Indirect immunofluorescence and flow cytofluorometry were used to study the binding of the monoclonal antibodies on A204-M7, A204, HeLa, Fcf2th-BILN, Fcf2th, and 940C3 cells. Comparisons of histograms (not shown) of the various cell lines are summarized in Table 3. Calculations of the ratio $(\phi - \phi_{auto})/(\phi_{FITC} - \phi_{auto})$ (ϕ is the mean fluorescence intensity per cell; see above) confirmed the

TABLE 3. Monoclonal antibody binding to different cell lines, measured by quantitative flow cytofluorometry

Antibody	Binding[a] to cell lines from:						
	Human origin			Dog origin		Monkey origin	
	A204-M7 (BaEV infected) (a)	A204 (uninfected)	HeLa (uninfected) (a)	Fcf2th-BILN (BaEV infected)	Fcf2th (uninfected) (a)	940C3 (M-PMV infected)	
		a b		a b		a b	
A6- 8	+	− 0.8	−	+ 7.8	−	− 1.1	
B12- 9	±	± 1.4	±	− 1.0	−	− 0.8	
C9-12	+	− 0.7	−	+ 2.2	−	− 1.0	
D12- 2	+	+ 10.7	+	− ND	−	− 1.1	
E4- 6	+	+ 10.4	+	− ND	−	− 1.1	
F12- 9	−	− 1.0	−	− ND	−	− 1.1	
B9-18	+	− ND[b]	−	ND ND	ND	ND ND	

[a] a, Qualitative results: comparison of histograms for antibody-treated cells and control cells as described in the text. +, Profile clearly displaced toward higher fluorescence intensities; −, identical profile; ±, slight increase in fluorescence intensity. b, Quantitative results: ratio of mean fluorescence intensity per monoclonal antibody-treated cell to that of control cell ($\phi - \phi_{auto}/\phi_{FITC} - \phi_{auto}$ [see text for a description of the variables]).
[b] ND, Not determined.

TABLE 4. Competitive binding of A6-8 and C9-12 antibodies of Fcf2th-BILN cells and of D12-2 and E4-6 antibodies on HeLa cells, studied by flow cytofluorometry[a]

First antibody (undiluted)	Second antibody (1:50 dilution)	FITCconjugate (1:20 dilution)	Mean fluorescence per cell[b]
—[c]	C9-12	Anti-IgG2b	2.4
A6-8	C9-12	Anti-IgG2b	1.5
—	A6-8	Anti-IgG2a	1.7
C9-12	A6-8	Anti-IgG2a	1.2
A6-8	—	Anti-IgG2b	1.1
C9-12	—	Anti-IgG2a	1.1
—	—	Anti-IgG2a or anti-IgG2b	1.0
—	D12-2	Anti-IgG2a	4.7
E4-6	D12-2	Anti-IgG2a	1.0
—	E4-6	Anti-IgM	4.9
D12-2	E4-6	Anti-IgM	3.1
E4-6	—	Anti-IgG2a	1.1
D12-2	—	Anti-IgM	1.0
—	—	Anti-IgG2a or anti-IgM	1.0

[a] The antibodies used were of the following classes: A6-8 and D12-2, IgG2a; C9-12, IgG2b; and E4-6, IgM.
[b] $\phi - \phi_{auto}/\phi_{FITC} - \phi_{auto}$.
[c] —, No antibody added.

rather subjective approach of histogram comparisons. Results in Table 3 show that A6-8 and C9-12 antibodies reacted with the BaEV-producing cell lines (A204-M7 and Fcf2th-BILN) but not with the corresponding virus-free control cells (A204 and Fcf2th, respectively). Negative reactions were also obtained with HeLa cells and with a monkey cell line (940C3) producing M-PMV. It should be noted that the binding of B9-18 antibody to the A204-M7 cells occurred with some variability from one experiment to another, suggesting that the corresponding epitope could be more or less expressed at the surface of the virus-producing cell. B12-9 antibody bound slightly to all human cell lines tested but did not react with cell lines of other species. Two other monoclonal antibodies, D12-2 and E4-6, bound very markedly to the human cell lines but not to the cell lines of dog or monkey origin. Two lines of evidence indicate that D12-2 and E4-6 antibodies were not directed against BaEV antigens. First, these antibodies bound to A204 cells as well as to the virus-producing A204-M7 cells. Second, no reaction was observed with dog thymus cells producing BaEV (Fcf2th-BILN cells). Finally, F12-9 antibody reacted with none of the cell lines.

We then turned to the question of the specificities of A6-8 and C9-12 antiviral antibodies. Did they recognize distinct or closely related epitopes? We were able to determine whether the two antibodies could compete with each other by taking advantage of the fact that A6-8 and C9-12 antibodies belonged to two different IgG subclasses: IgG2a and IgG2b, respectively. Indirect immunofluorescence provided an easy means to selectively reveal the presence of one monoclonal antibody by use of appropriate FITC reagents. To saturate the corresponding epitope, 0.5×10^6 BaEV-producing Fcf2th-BILN cells were first incubated for 30 min at 4°C with 100 μl of one of the two undiluted monoclonal antibodies (e.g., A6-8). After two washings, cells were treated for 30 min at 4°C with 100 μl of a 1:50 dilution of the second antibody (C9-12 in our example). Cells were again washed twice and incubated for 30 min at 4°C with 100 μl of a 1:20 dilution of FITC-conjugated goat IgG anti-mouse IgG2b.

The two extreme possibilities which we predicted are as follows. If both antibodies recognized the same epitope, adsorption of A6-8 would inhibit C9-12 binding, and FITC reagent specific for C9-12 would not stain the cells. On the other hand, if the two antibodies recognized two different epitopes situated sufficiently far from each other, fixation of one antibody would not interfere with binding of the second antibody. In our example, this would lead to cellular fluorescence. As shown in Table 4, pretreatment with one of the monoclonal antibodies reduced markedly the binding of the second antibody, without complete abolition, however. It is important to note that the results did not depend on which antibody was added first. Similar results were obtained on A204-M7 cells. We also observed a competition between the anticellular antibodies D12-2 and E4-6 on HeLa cells, particularly when the IgM E4-6 was added first (Table 4).

Inhibition of syncytium formation with mono-

TABLE 5. Neutralizing activity of monoclonal antibodies on syncytium-forming units of BaEV and M-PMV[a]

Antibody	BaEV		M-PMV	
	No. of syncytia[b]	% of surviving virus	No. of syncytia[b]	% of surviving virus
A6-8	24	29	40	133
B9-18	88	104	ND[c]	
C9-12	0	0	41	137
D12-2	94	112	ND	
E4-6	90	107	ND	
F12-9	105	125	ND	
None	84	100	30	100

[a] A 1,800-μl sample of virus preparation was mixed for 1 h at 37°C with 200 μl of antibody preparation, and 200-μl portions were distributed into eight wells containing monolayers of RSa cells.
[b] Number of syncytia per well (mean of eight wells; standard deviations were 4 to 12%).
[c] ND, Not determined.

clonal antibodies. The ability of BaEV to induce multinucleated foci (syncytia) in RSa monolayers provided an assay to investigate the capability of monoclonal antibodies to inhibit a viral function. The neutralizing activity of monoclonal antibodies diluted 1:20, after 1 h of contact at 37°C with BaEV or M-PMV, is shown in Table 5. The syncytium-forming units of BaEV were completely neutralized by the culture fluid of clone C9-12 and were reduced to 29% of the original amount after treatment with culture fluid from clone A6-8. None of the antibodies neutralized M-PMV. With this virus, the number of syncytia obtained after contact with the medium containing antibodies was even greater than the number obtained after contact with tissue culture medium alone. Although the difference was significant, we have no explanation for this phenomenon. Figure 1 illustrates the efficiency of neutralization by clone C9-12 at various dilutions. Complete inhibition of syncytium formation was still obtained at a 1:100 dilution. A 50% inhibition was observed at a 1:200 dilution.

Although D12-2 and E4-6 antibodies did not inhibit BaEV syncytium formation, treatment of RSa cells with a 1:5 dilution of these antibody preparations for 2 h at 37°C followed by washing of the cells resulted in the inhibition of syncytium formation by BaEV (Table 6). A similar treatment of RSa cells did not preclude syncytium formation by M-PMV.

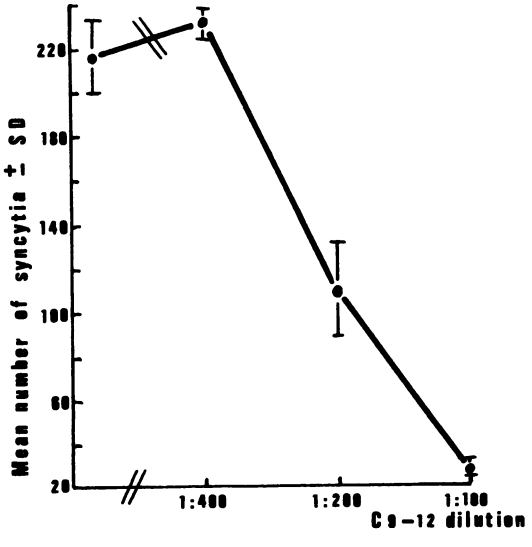

FIG. 1. Inhibition of BaEV syncytium formation by different concentrations of C9-12 antibody. Approximately 200 BaEV syncytium-forming units were mixed for 1 h at 37°C with various dilutions of C9-12 antibody or with medium alone and then inoculated to RSa monolayers. Syncytia were counted after 48 h in a CO_2 incubator.

TABLE 6. Effect on syncytium formation by BaEV of treatment of RSa cells with monoclonal antibodies[a]

Antibody	No. of BaEV syncytia[b]	% of control
A6-2	27 ± 7	104
A6-8	25 ± 9	96
C9-12	27 ± 5	104
D12-2	3 ± 2	12
E4-6	6 ± 2	23
None	26 ± 8	100

[a] Each well containing RSa cell monolayers was treated for 1 h at 37°C with 200 μl of a 1:5 dilution of antibody preparation. Cells were then washed, and 200 μl of the virus preparation was added.

[b] Number of syncytia per well (mean ± standard deviation of eight wells).

Immune precipitation of viral proteins. Monoclonal antibodies produced by hybrid cell lines in culture were examined in radioimmune precipitation assays with Nonidet P-40 lysates of [^{3}H]leucine-labeled BaEV. Monoclonal antibody from F12-9 was found to precipitate selectively an antigen with a molecular weight of 18,000 (Fig. 2). By comparison with the well-studied polypeptides from other type C retroviruses, this protein with a molecular weight of 18,000 could be analogous to protein p15C or p15E from MuLV (19) or to protein p16 identified in BaEV (24).

The three antiviral antibodies A6-8, B9-18, and C9-12 precipitated exclusively a polypeptide with a molecular weight of 78,000, as shown on the densitometer tracing of Fig. 2. The proteins recognized by the anticellular antibodies have not yet been identified.

DISCUSSION

We obtained monoclonal antibodies against BaEV proteins by fusion of mouse myeloma cells with splenocytes from mice immunized with semipurified preparations of virus.

Three types of experimental results indicated that the antibodies produced by the clones A6-8 and C9-12 were directed against BaEV proteins. These immunoglobulins bound to the surfaces of virus-producing cells exclusively, as shown by cytofluorometric analysis of living cells and by RIA of fixed cells. They also specifically neutralized the syncytium-inducing capacity of BaEV. It must be noted that the presence of complement was not required for this neutralization, in contrast to the results described for the Friend MuLV-neutralizing antibodies (6). It has been speculated by Fine and Arthur (9) that neutralizing antibodies for simian retroviruses are directed against the gp70 glycoprotein of the viral envelope. In agreement with this, all of the monoclonal antibodies which have been found

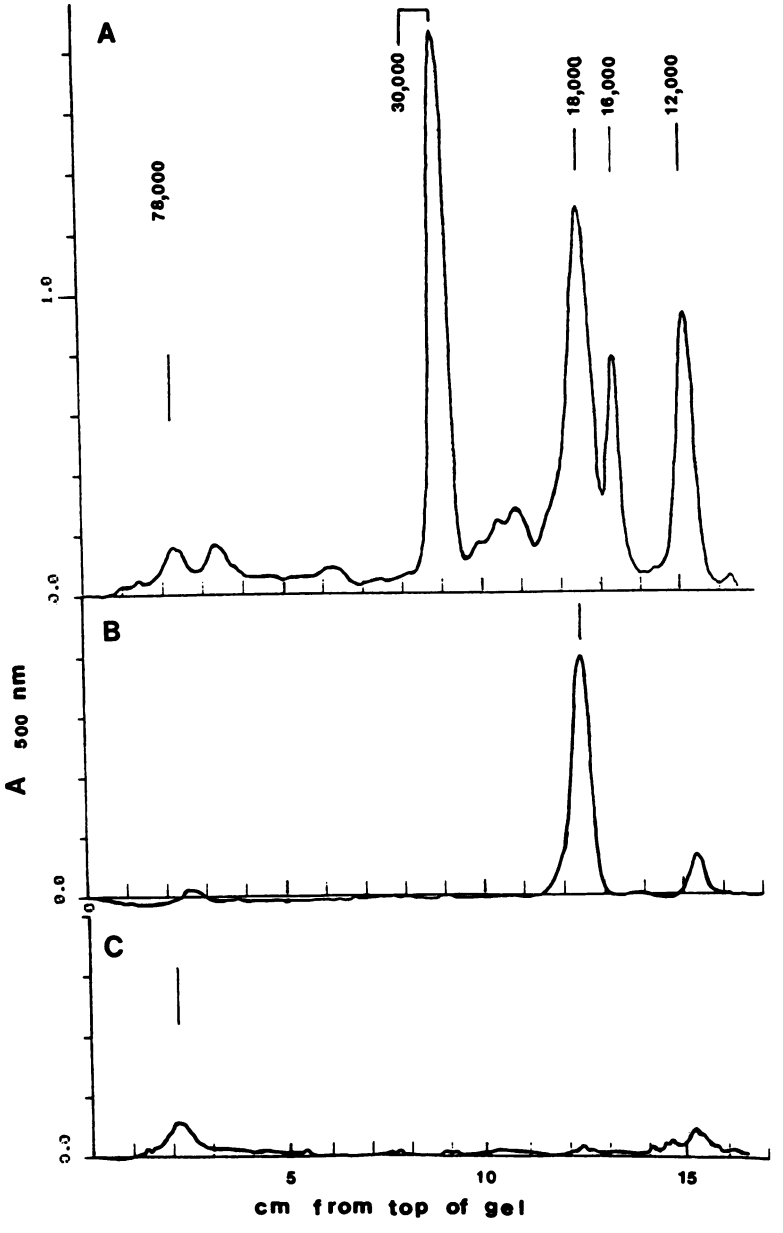

FIG. 2. Densitometric scans of polyacrylamide gels after electrophoresis and fluorography. (A) [^{3}H]leucine-labeled BaEV used as antigen. (B) Immune precipitate with F12-9 antibody (a polypeptide with a molecular weight of 18,000). (C) Immune precipitate with A6-8 antibody (a polypeptide with a molecular weight of 78,000). Results were similar for C9-12 and B9-18 antibodies. The minor peak of low molecular weight was visible with all of the precipitates and probably contained breakdown products. Exposure time was 3 weeks at −70°C. Tracings were made in a Beckman DU-8 spectrophotometer. A_{500} nm, Absorbance at 500 nm.

to neutralize the infectivity of murine retroviruses were reactive with determinants on gp70 (6, 21), and we conclude that A6-8 and C9-12, which precipitate a polypeptide with a molecular weight of 78,000, are directed against the envelope glycoprotein of the virion. We suggest that the combining site for B9-18 is located in another region of the same polypeptide, as this antibody did not affect syncytium formation by BaEV.

Cytofluorometric analysis showed that C9-12 antibody competed with A6-8 antibody for binding to A204-M7 cells and also to Fcf2th-BILN cells. The reverse competition was similarly observed. This indicates that the determinants

recognized by these two antiviral antibodies are situated close to each other on the surfaces of the infected cells.

RIAs with culture fluid from clone F12-9 indicated that this antibody was also directed against a viral protein which, however, was not detectable on the surface of the infected cell by cytofluorometric assay and which was not implicated in syncytium induction on RSa cells, since this property of BaEV was not inhibited by antibody from clone F12-9.

The immune complex formed with [^{3}H]leucine-labeled virus and F12-9 culture fluid revealed a single band migrating with an apparent molecular weight of 18,000 in a polyacrylamide gel. Whether this polypeptide is analogous to the p16 protein associated with the 70S genome in the virion (23) or whether it can be compared to p15C or p15E protein from murine retroviruses must be further studied.

By contrast, it is clear that the antibodies secreted by clones D12-2 and E4-6 are directed against cellular components. Our preliminary results indicated that the cell determinants recognized by these antibodies may be situated within the receptors for BaEV on human cells. This assumption is derived from the observation that treatment of the RSa cells with these antibodies precluded the further induction of syncytia by the virus, although these antibodies were inactive on the virus itself. However, the canine cells, which also possess receptors for BaEV, did not bind to these two antibodies, as observed by cytofluorometry. It may be assumed that differences exist among the antigenic determinants of the site of attachment for a given virus on cells from different animal species. The characterization of these cell sites will be pursued.

For instance, it will be interesting to investigate whether these cell determinants bear some similarities to the 55,000-dalton membrane glycoprotein which has been shown to be associated in high concentration with several rodent retroviruses (22). Such determinants may have been present in the virus preparations utilized for the immunization of mice and for the selection of antibodies in the present report.

ACKNOWLEDGMENTS

We thank Marie-Louise Blondiau and Marcel Jacques for their expert technical assistance and Jeannine Herinckx and Leopold Tack for typing of and illustration for the manuscript.

This work was supported by grants from Fondation Hoguet, Fonds de la Recherche Scientifique Médicale, and Fonds de Cancérologie Caisse Générale d'Epargne et de Retraite. R.O. is an "aspirant du Fonds National Belge de la Recherche Scientifique."

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