ALTERATION OF IMMUNOGLOBULIN PHENOTYPE IN CELL CULTURE-ADAPTED LINES OF TWO MOUSE PLASMACYTOMAS*

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As commonly understood, clonal hypotheses for immunoglobulin (Ig) diversity require that antigens selectively stimulate different antibody cell clones and that the daughter cells of each clone produce antibodies having identical antigen-combining sites (1–3). In support of this view is the fact that in mice neoplastic Ig-producing cell lines (plasmacytomas) usually retain their individuality and homogeneity of Ig phenotype after repeated transplantation in vivo (4); also, the Ig's of many plasmacytomas have been shown to be specific for various antigens common to the environment of the mouse (5). In these respects, plasmacytomas seem to share the same properties as inferred for single clones of experimentally induced antibody-forming cells (6–8). However, the stability of Ig phenotype in mouse plasmacytomas is not without exception. These exceptions mostly take the form of defective Ig-producing cell lines that secrete only light (L)[1] chains or that do not secrete an Ig at all (nonproducers) (9–11).

In contrast to defective Ig-producing cells, we have obtained two plasmacytoma cell lines in culture (104-76 and 352-57) that produce intact Ig's containing heavy (H) and L chains that differ from those produced by the "parent" plasmacytomas (MOPC 104E and MOPC 352). In this respect and as a matter of convenience, we refer to 104-76 and 352-57 as variant Ig-producing cell lines. The central question raised by these results is whether these cell lines represent clonal variants of MOPC 104E and MOPC 352 or secondary tumors of host origin. As clonal variants, it would be of interest to know whether this apparent variable Ig expression has meaning for antibody diversity. As secondary host tumors, one would like to know the mechanism of tumor induction.

Materials and Methods

Allotypes of Different BALB/c Congenic Mice.[2] BALB/c mice congenic for different Ig allotypes were used exclusively in these studies. In Table I, the H-chain allotypes of these congenic strains

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[1] *Abbreviations used in this paper:* BME-199, 80% Eagle's basal medium and 20% medium 199 supplemented with 20% heat-inactivated FCS, 3 mM glutamine, sodium bicarbonate, and antibiotics; H, heavy; L, light; MOPC, mineral oil-induced plasmacytomas.

[2] The CB and CAL congenic mouse strains originated in the laboratory of Dr. Michael Potter, as did all of the mouse plasmacytomas used in this study.

TABLE I
Allotypes of Different BALB/c Congenic Mouse Strains

Mouse strain*	Allotype determinants of the H-chain linkage group‡
BALB/c	$A^{12,13,14}$ F^{19} $G^{1,6,7,8}$ $H^{9,11}$
CAL-20	$A^{13,17}$ F^{19} $^4G^{6,7,8}$ H^4
CB-17	A^{15} F^{8} $^2G^-$ $H^{9,16}$

* BALB/c mice congenic for different Ig allotypes are designated CAL for those having the allotype of the AL/N mouse strain and CB for those having the allotype of the C57BL/Ka mouse strain (5). The number after the hyphen refers to how many backcross generations preceded the derivation of each congenic strain.

‡ A, F, G, and H refer to the Ig classes IgA, IgG_1, IgG_{2a}, and IgG_{2b} (4). These Ig classes have been denoted Ig-2, Ig-4, Ig-1, and Ig-3 elsewhere (12). The assigned allotypic determinants are numbered according to Potter and Lieberman (4).

are listed. A, F, G, and H refer respectively to the Ig classes IgA, IgG_1, IgG_{2a} and IgG_{2b} which are distinguished allotypically according to the assigned numbers of Potter and Lieberman (4, 5).[3] The abbreviated designation of BALB/c congenic mice that carry the Ig genes of the C57BL allotype (CB mice) or the Ig genes of the AL/N allotype (CAL mice) is according to the notation used by Potter (5).

Ig Preparations from Plasmacytoma-Bearing Mice.[2] Transplantable lines of mineral oil-induced plasmacytomas (MOPC) of BALB/c or of CB mice were stored in liquid nitrogen freezers. For routine propagation, plasmacytomas were transplanted subcutaneously. Depending on the allotype of the mouse in which the tumor arose, each plasmacytoma used in this study (unless otherwise stated) was grown in congenic BALB/c mice having a different allotype than that of the tumor Ig.

$(NH_4)_2SO_4$ precipitation and DEAE-cellulose chromatography (at 0.02 M PO_4, pH 7.4) were the methods (13) used to obtain 7S Ig from the pooled sera of plasmacytoma-bearing mice. Papain digestion of 7S Ig (14) yielded Fc and Fab fragments that were separated over DEAE-cellulose columns by means of a linear salt gradient (0.005 M PO_4 to 0.3 M PO_4, pH 8.0) (15).

Salt-precipitated IgM of MOPC 104E and a variant of MOPC 104E (104-76) in 0.1 M PO_4, pH 7.4 were fractionated over a 1.5 × 90-cm column of Bio-Gel A-1.5m Agarose (Bio-Rad Laboratories, Richmond, Calif.) Iodination of IgM was done according to the chloramine T method of Hunter and Greenwood (16); the free ^{125}I was separated from the labeled protein by passage over a mixed bed of ion-exchange Resin (Amberlite IR 4B & Bio-Rad AG 1 × 8) layered on top of a G-50 Sephadex column.

Antisera. Goat antiserum specific for mouse IgM was purchased from Maloy (Springfield, Va.). This antiserum was absorbed with the lambda-containing IgG_{2a} from the HOPC-1 plasmacytoma in order to remove residual anti-lambda (λ) specificity. For this as well as other absorptions, Ig antigens were coupled to Sepharose beads according to the method of Axen et al. (17). Rabbit antiserum directed against mouse L chains of either the λ or kappa (κ) classes was given to us by Dr. Martin Weigert of this institute.

Antiallotype antibodies were produced in C57BL/6 and AL/N mouse strains after the injection of agglutinates of rabbit red cell ghosts and 7S Ig of C3H/HeJ mice (18). Antisera were tested by precipitation in micro-Ouchterlony.

C57BL/6 antiserum obtained during the first 6 mo of the immunization regimen was specific for $G^{6,7,8}$ and F^{19} allotypic determinants, but in later months (9–12 mo), most of the surviving mice switched entirely to the production of anti-F^{19} antibodies. Monospecificity of C57BL/6 antisera to

[3] These Ig classes have been denoted by Herzenberg et al. (12) as Ig-2, Ig-4, Ig-1, and Ig-3, respectively. Here, individual allotypic determinants are designated by a second number (e.g., Ig-1.1).

either $G^{6,7,8}$ or F^{19} was assured after absorption with Sepharose-conjugated Ig of the appropriate plasmacytomas as well as with Sepharose-conjugated Fab fragments derived from the 7S Ig fraction of C3H/HeJ mice immune to rabbit ghosts.

The AL/N antisera gave precipitin bands to BALB/c and C57BL 7S Ig. When this antiserum was tested against Ig preparations from different plasmacytomas, it gave precipitin bands of identity to IgG_{2a} and IgG_{2b}, but it failed to precipitate IgG_1. For the purpose of this test, the tumors producing IgG_{2a} (MOPC 173, LPC-1), IgG_{2b} (MOPC 195, MOPC 352) and IgG_1 (MOPC 31_c) were all grown in BALB/c congenic mice (CA1-20) that have the same Ig allotype as AL/N mice. On the basis of these results, we have tentatively designated the AL/N antiallotype serum as anti-G^aH^a.

In contrast to the specificity of mouse antiallotype sera for Ig determinants on the Fc fragment, we raised a BALB/c antiserum against determinants found on the Fab fragments of MOPC 352 Ig. This BALB/c antiserum was absorbed to monospecificity with Sepharose-conjugated Fc fragments of MOPC 352 Ig, after which it failed to precipitate normal Ig of BALB/c, AL/N or C57BL mouse strains; nor did this antiserum precipitate any of the Ig preparations from the many plasmacytomas tested. Hence, we refer to this antisera as anti-352 Fab or anti-352 idiotype.

Tissue Culture Medium. Cells were adapted to culture in a medium composed of 80% Eagle's basal medium and 20% medium 199 supplemented with 20% heat-inactivated fetal calf serum (FCS), 3 mM glutamine, sodium bicarbonate, and antibiotics (BME-199). After complete adaptation to in vitro growth, tumor cells were usually cultured in Eagle's minimal essential medium supplemented with 10% FCS, 3 mM glutamine, sodium bicarbonate, and antibiotics (MEM).

Adaptation of Plasmacytomas to Culture. Tumor cells were adapted to culture by an animal passage technique (19, 20); this procedure of alternate growth in vitro and in vivo was continued until tumor cells were able to proliferate to high density in culture. To facilitate the handling of tumor cells, plasmacytomas were injected intraperitoneally into each new host mouse to induce ascites. The ascites fluid (2–3 ml) which contained tumor cells, erythrocytes, peritoneal macrophages and other reticulum cells, was aseptically aspirated. Red cells were lysed after two successive periods (1 min each) of hypotonic shock in distilled water. The washed cells were resuspended in BME-199, placed in 75-cm^2 plastic flasks (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) or in Spinner bottles (200 ml vol) and incubated at 37°C in an atmosphere of 95% air and 5% CO_2. Cultures were observed daily and usually fed thrice weekly. Tumor cells were considered fully adapted to culture after five successive in vitro passages and at such time were frozen and stored in liquid nitrogen. Cell counts were made in a hemacytometer and the number of viable cells scored on the basis of their ability to exclude eosin.

Results

Cell Growth of an IgG_{2a}-Producing Plasmacytoma During Adaptation to Culture. To select for plasmacytoma cells that can grow in culture, it is necessary to place tumor cells under alternating conditions of in vitro and in vivo growth (20). The results of Fig. 1 seem to reflect this kind of cell selection for MOPC 173 (173-39) after it was placed in spinner culture for the third time. Here, in a culture medium maintained at 200 ml, the cell concentration and cell viability (by eosin exclusion) were followed for over a 200-day period. After 17 days in culture, the number of tumor cells seeded (6.0×10^8) declined to less than 3×10^4 living cells, i.e., only 0.05% of the cells counted were viable. However, over the next 20 days, the number of viable cells increased exponentially to greater than 10^7 cells. After two dilutions of the culture with fresh medium, the number of cells remained relatively static until day 55, at which time 173-39 began to show signs of being adapted. From day 65 onward, the cell growth was exponential in the range of 10^4–10^6 cells/ml with a calculated doubling time of 19 h. When culture densities exceeded 10^6 cells/ml, we found that the cell growth slowed considerably and that the fraction of viable cells dropped below 90%. After 80 days in Spinner culture 173-39 was also placed in culture flasks where it continues to produce IgG_{2a} in its 20th serial passage.

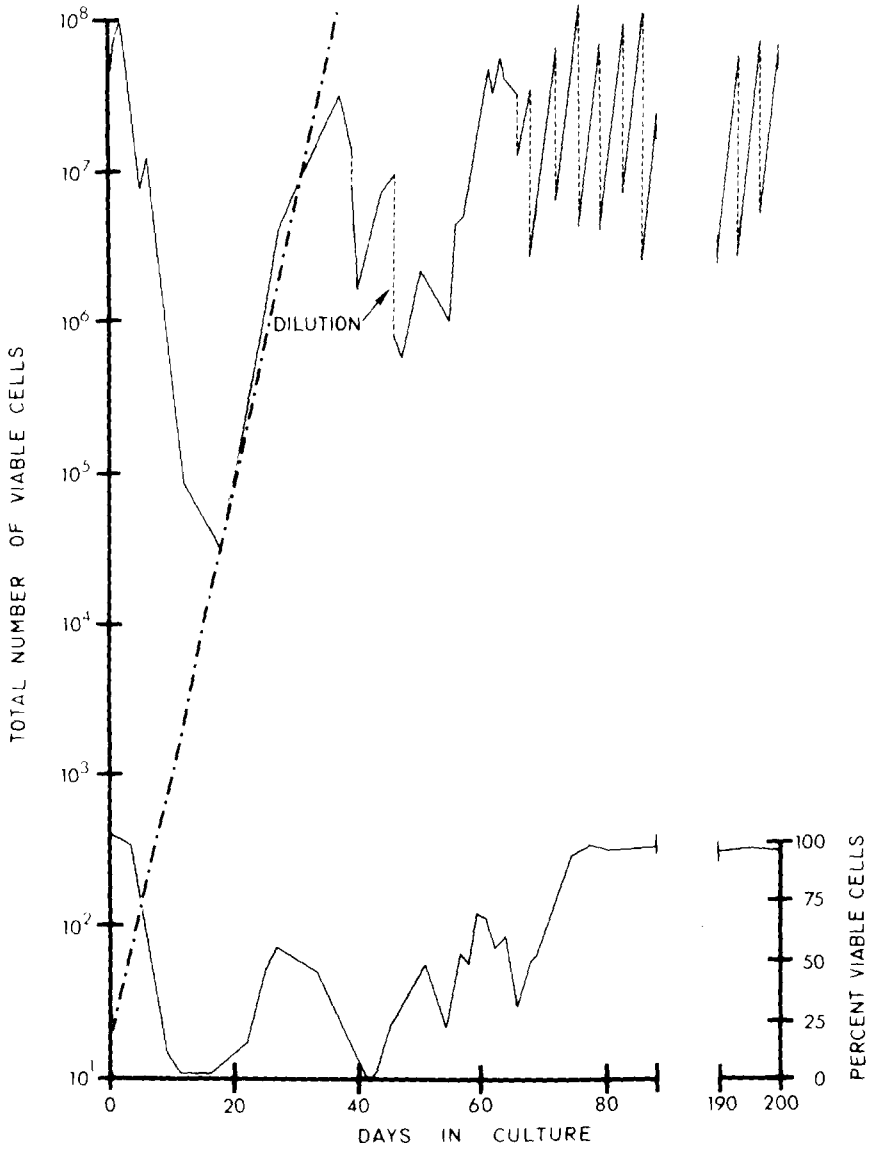

FIG. 1. Growth behavior of MOPC 173 (173-39) during adaptation to culture in BME-199 containing 20% fetal calf serum. After 173-39 cells were placed in spinner culture for the third time, the cell concentration and cell viability (cells that did not stain with eosin) were recorded for over 200 days. The 173-39 cell population was fed periodically and when cell densities approached 10^6 cells/ml, the culture was "split" (decanted) and diluted with new medium as indicated (---). Throughout the entire period of adaptation, the culture volume was maintained at 200 ml.

Extrapolating the initial growth curve of 173-39 (see Fig. 1) back to zero time shows that less than 50 cells gave rise to the 173-39 cell line, i.e., only about one in 10^7 cells of the starting population could grow in culture. Such rare cells (10^{-7}) must be different from most cells, i.e., they must be variant cells. And the inclusion of an altered Ig phenotype among such cells could explain the variant

Ig-producing cell lines that we observed in two of six different Ig-producing plasmacytomas adapted to culture: PC-3 (IgG_{2a}), TEPC-15 (IgA), MOPC-173 (IgG_{2a}), PC-1171 (IgG_1 of an NZB mouse), MOPC-104E (IgM), and MOPC-352 (IgG_{2b} of C57BL allotype). The two variant cell lines are described next.

Detection of a MOPC 104E Variant in Culture. The first plasmacytoma variant (104-76) was found in MOPC 104E, an IgM-producing tumor. IgM molecules of MOPC 104E are distinguished by their λ-light chains and by their ability to bind α1-3 Dextran (21). Since we did not examine 104-76 for Ig markers until after it was completely adapted to culture, we were surprised to find that 104-76 produced IgM molecules that were different than those of the MOPC 104E line. As can be seen in Table II, 104-76 produces IgM molecules containing κ- instead of λ-light chains; further, the IgM of 104-76 does not have antibody specificity for α1-3 Dextran. By testing frozen serum pools of MOPC 104E-bearing mice of earlier transplant generations, we were able to determine that the MOPC 104E tumor stopped synthesizing IgM molecules with Dextran-binding specificity around the 64th transplant generation.

The basis for the data of Table II is as follows: both the IgM of 104-76 and MOPC 104E (grown-up from frozen ampoules of transplant generation 50) were twice-fractionated separately over a 1.5 × 90-cm column of BioGel 1.5A to give nearly coincident peaks immediately after the void volume (this is illustrated in Fig. 2). When the protein in these peaks was concentrated and run against the appropriate antisera, we observed the precipitin patterns shown in Fig. 3. Here, the Ig's of different plasmacytomas (as arranged in the Ouchterlony wells) serve to demonstrate the specificity of the antisera as well as that of the 104-76 Ig. Other than what has already been summarized in Table II, these results with gel-fractionated IgM make it clear that the κ- and λ-chains of 104-76 are part of the same molecule.

Comparative immunoelectrophoresis between the IgM of MOPC 104E and 104-76 revealed yet another difference (see Fig. 4), namely, that the IgM of 104-

TABLE II

Detection of a MOPC 104E Variant (104-76) in Culture

MOPC 104E transplant generation	Generation used for culture	Animal passage*	Culture passage*	Ig markers‡			
				μ	λ	κ	Dex
51–55				+	+	–	+
56–64				NT	NT	NT	+
65–70				NT	NT	NT	–
71–75				NT	NT	NT	–
76	104-76	First		NT	NT	NT	–
	104-76	Second		NT	NT	NT	–
	104-76		Fifth	+	–	+	–
	104-76		Tenth	+	–	+	–

* Animal passage and culture passage refer to the number of times 104-76 was alternately grown in mice or serially passaged in culture.

‡ The presence or absence of IgM heavy chains (μ), of λ– or κ– light chains, and of antibody specificity for α1-3 Dextran (Dex) is indicated with a plus (+) or minus (–). NT denotes not tested.

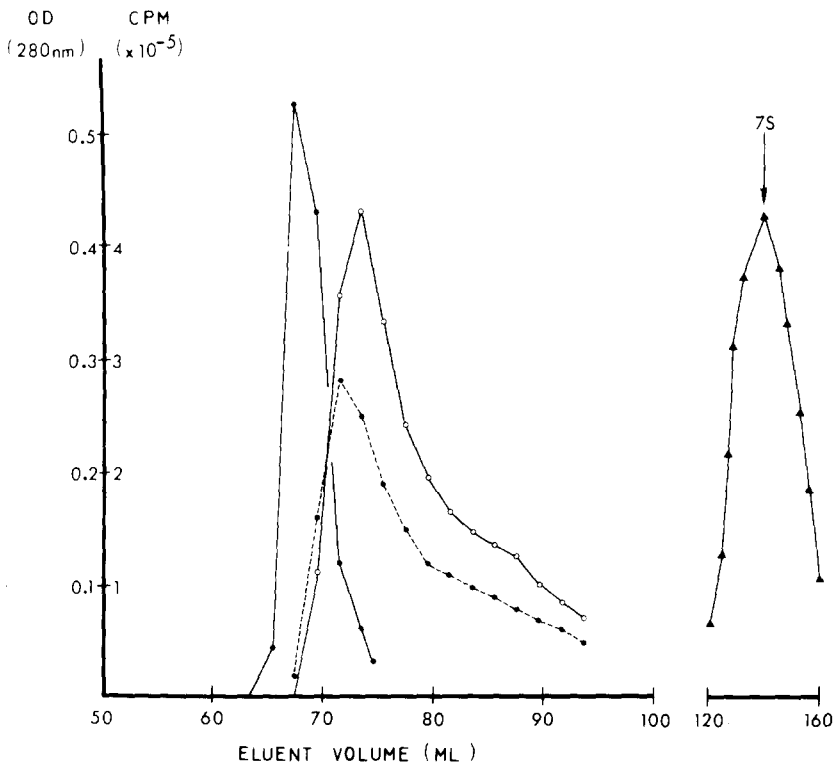

FIG. 2. Gel-filtration of IgM from MOPC 104E and 104-76 over a 1.5 × 90 cm column of BioGel A-1.5m agarose. The IgM in the pooled sera of tumor-bearing CB-17 mice was first precipitated, concentrated and fractionated over the BioGel column (conditions: 1 M NaCl in 0.1 M PO_4, pH 7.4). 1-ml portion of each fractionated IgM preparation was recycled separately over the same column to give the elution pattern seen: 0.2 mg of [^{125}I]104E, specific activity, 5 × 10^4 cpm/μg; (●---●), 17 mg of 104-76 (○—○); and as a marker for the column void volume, 0.2% Blue Dextran (●—●). A comparable coincidence of elution profile was obtained with a mixture of [^{125}I]104E IgM and 104-76 IgM. The eluent volume of IgG_{2a} from the LPC-1 plasmacytoma served as a 7S Ig marker (▲—▲).

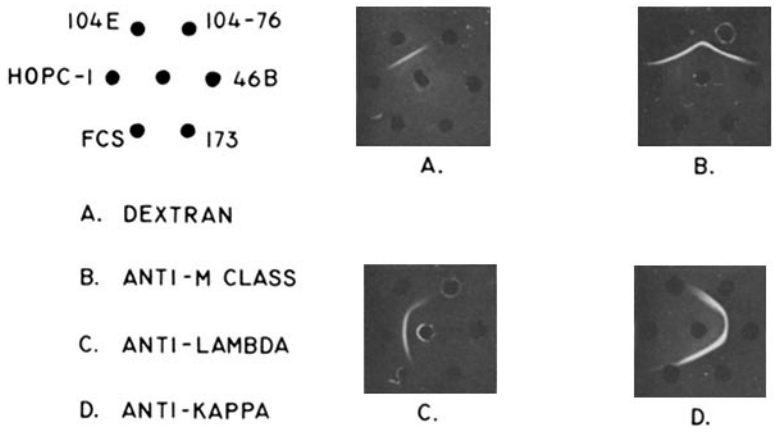

FIG. 3. Comparative analysis of the antigenic specificities of gel-fractionated IgM from Fig. 2. As indicated, the outer wells contained the following purified Ig preparations: 104-76 (2.0 mg/ml); 104E (1.0 mg/ml except Ouchterlony C, 0.15 mg/ml); MOPC 46B (κ-chains at 0.1 mg/ml); MOPC 173 ($G^{6,7,8}$ at 0.2 mg/ml); HOPC-1 (λ-containing $G^{6,7,8}$ at 0.2 mg/ml) and FCS (20% fetal calf serum). The center wells had the following: (A) dextran (1.0 mg/ml); (B) goat anti-IgM; (C) rabbit antimouse λ-chain; and (D) rabbit anti-κ chain.

76 does not migrate toward the anode as does the IgM of MOPC 104E. The lack of migration by 104-76 IgM cannot simply be attributed to the light chain substitution of κ for λ since the κ-containing IgM of TEPC-183 migrates as does the IgM of MOPC 104E (Fig. 4 C). This fact and the results of other experiments (to be published separately) showing the IgM of MOPC 104E and 104-76 to be dramatically different in their susceptibility to papain digestion and different in their μ-chain (H) antigenicity (as determined with a solid-phase radioimmune assay), but having μ-chains of indistinguishable size according to gel electrophoresis of the separated μ-chains in polyacrylamide containing 7% sodium dodecyl sulfate, lead us to suspect that 104-76 may be expressing altered μ-chains or a μ-chain class that is different than that of MOPC 104E.

Another point to be made from the results of Fig. 4 is the noted absence of the 104-76 variant in the MOPC 104E parent line. Although not shown here, neither were we able to detect the variant IgM in tumor-bearing sera of later transplant generations (71-75). Thus, the loss of precipitin bands to Dextran after transplant generation 64 was not accompanied by a detectable gain of 104-76 IgM.

Detection of a MOPC 352 Variant in Culture. A second plasmacytoma variant (352-57) was found in MOPC 352 which originated in a BALB/c congenic

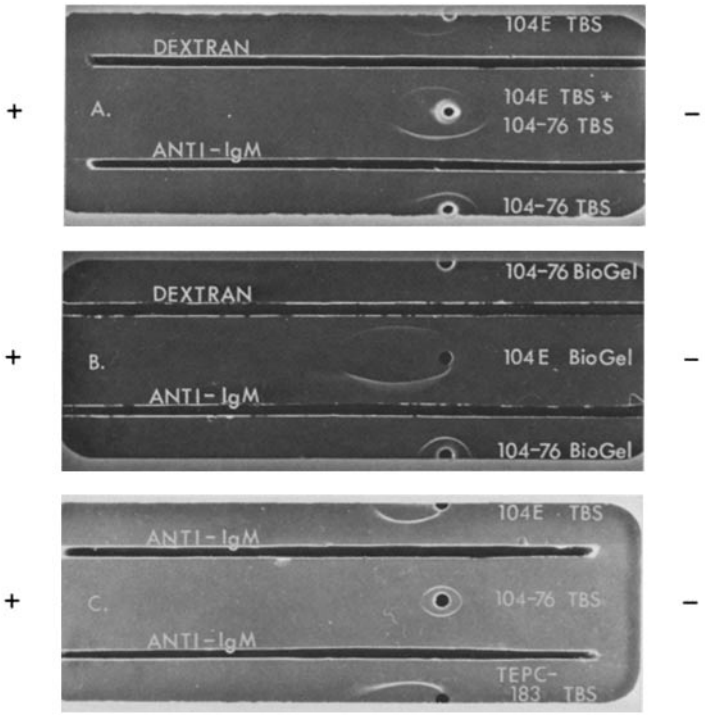

FIG. 4. Comparative immunoelectrophoresis of IgM-producing plasmacytomas (conditions: 9 V/cm for 60 min in 1% agarose containing 0.05 M barbital buffer, pH 8.6), Dextran (5 mg/ml) or goat anti-IgM sera were placed in the troughs as indicated. The wells contained tumor-bearing serum (TBS) of mice in which either MOPC 104E, 104-76 or TEPC 183 was growing. Also included were IgM preparations of MOPC 104E (8.2 mg/ml) and 104-76 (17 mg/ml) that were obtained by fractionation over a 1.5 × 90 cm column of BioGel A-1.5m agarose.

mouse (CB-6) that was homozygous for a marker chromosome containing Ig genes of the C57BL allotype. The IgG_{2b} produced by MOPC 352 can be distinguished serologically from IgG_1 on the basis of its H^9 allotype. Further, MOPC 352 can be distinguished from all other plasmacytomas by means of a BALB/c antiserum specific for antigenic determinants found on the Fab fragments of MOPC 352 Ig (352 Fab marker).

The adaptation of MOPC 352 to culture, as it was alternately grown in vitro and in vivo (in BALB/c mice) was followed by means of the 352 Fab marker. The results of Table III indicate that the persistence of this marker faded during the adaptation process such that the fully adapted 352-57 lost the 352 Fab marker completely. We were inclined to accept 352-57 as a nonproducer but after screening the tissue culture fluid against various antisera, we found that 352-57 was producing an Ig bearing the F^{19} allotype.

Other than testing culture fluids for the 352 Fab marker, the basis for Table III is as follows: 352-57 and MOPC 352 (from the same lot of frozen ampoules from which 352-57 was originally started) were grown in BALB/c, CB-17 mice, and CAL-20 mice; two transplant generations later, we tested the sera of the second tumor-bearing hosts for the presence of F^{19}, H^9, and the 352 Fab marker. The results are shown in Fig. 5. The absence of F^{19} precipitin bands in the sera of CB-17 mice bearing MOPC 352 (Ouchterlony A) held true even after concentrating the tumor Ig fivefold.

Discussion

We have presented evidence for the selection of variant plasmacytoma cells in tissue culture. The action of selection was first indicated by the growth kinetics of 173-39 during its adaptation to culture, the inference being that very few tumor cells in the starting population (10^{-7}) can give rise to a cultured cell line, and secondly by the detection of two variant Ig-producing cell lines (104-76 and 352-57) in cultures adapted from transplanted lines of MOPC 104E and MOPC 352. What follows is a discussion of possible explanations for the presence or occurrence of variant Ig-producing cells in a given plasmacytoma cell line.

TABLE III

Detection of a MOPC 352 Variant (352-57) in Culture

MOPC 352 transplant generation	Generation used for culture	Animal passage*	Culture passage*	Ig markers‡		
				F^{19}	H^9	352 Fab
48				−	+	+
54				NT	NT	+
57	352-57	First		NT	NT	+
	352-57	Second		NT	NT	±
	352-57		First	+	−	−
	352-57		Fifth	+	−	−

* Animal passage and culture passage refer to the number of times 352-57 was alternately grown in mice or serially passaged in culture.

‡ The presence or absence of different Ig heavy-chain allotypes (F^{19}, H^9) and of the 352 Fab marker is indicated with a plus (+) or minus (−). NT denotes not tested.

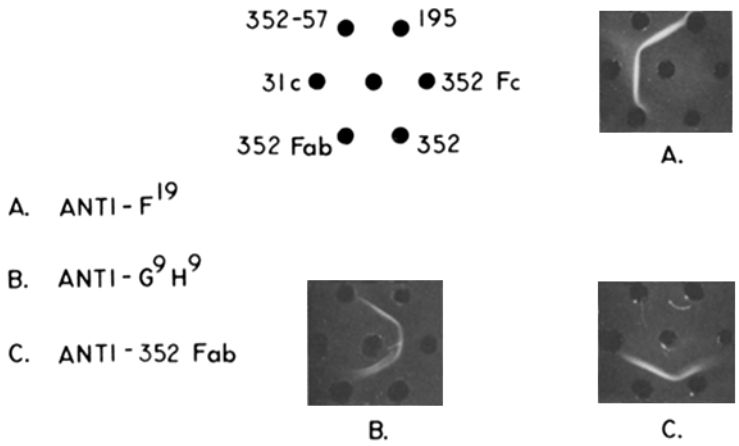

FIG. 5. Comparative analysis of the antigenic specificities of Ig produced by MOPC-352 and 352-57. As indicated, the outer wells contained tumor-bearing sera of MOPC 195 ($H^{9,11}$), MOPC 31c(F^{19}) and 352-57 grown in CB-17 (Ouchterlony A), in CAL-20 mice (Ouchterlony B), and in BALB/c mice (Ouchterlony C); and purified preparations of 352 (0.2 mg/ml), 352 Fc (0.1 mg/ml), and 352 Fab (0.2 mg/ml). The mouse antiallotype sera placed in the center wells was (A) C57BL/6 anti-F^{19}; (B) AL/N anti-G^9H^9; and (C) BALB/c anti-352 Fab.

The detection of variant Ig-producing plasmacytoma cells cannot be attributed to cross-contamination of 104-76 and 352-57 with other transplantable plasmacytomas. MOPC 104E was the only IgM-producing tumor in our laboratory during the adaptation of 104-76. Further, the BALB/c plasmacytoma (MOPC 31) that we regularly carry and use as representative of the IgG_1 allotype (F^{19}) produces an IgG_1 that has a strikingly different isoelectric-banding pattern than the F^{19} globulin of 352-57 (our unpublished results). Neither is there any evidence for contamination of our frozen tumor bank since for retesting purposes, we grew-up the MOPC 104E and MOPC 352 from the same lots of frozen ampoules that were used in the 104-76 and 352-57 adaptation series (Tables II and III).

Another possibility is that the parent tumor is biclonal so that 104-76 and 352-57 may have been present in MOPC 104E and MOPC 352 from the time of original tumor induction. Three arguments can be made against this view. First, if the division rates of two incipient clones at the time of induction and thereafter were to differ by only 5%, then the frequency of tumor cells from the slower dividing clone would be of the order of 10^{-12} after 50 transplant generations. This assumes a conservative 15 cell divisions per transplant generation, impartial host mice and equal selective value for the growth of each cell line over 50 transplant generations. That these conditions would be fulfilled by chance in two of six Ig-producing plasmacytomas that we successfully adapted to culture is improbable. Secondly, MOPC 352 was induced in a CB-6 mouse that was bred to exclude the BALB/c allotype.[4] Therefore, we would not expect to have a biclonal tumor of mixed allotypes (352-57 produces IgG_1 of the BALB/c allotype). And thirdly, of the thousand or more BALB/c plasmacytomas that

[4] Potter, M. Personal communication.

have been typed, only a rare few produce IgM.[4] This makes it highly improbable that 104-76 IgM is a biclonal contaminant of MOPC 104E.

Two interesting explanations for the present observations remain, one being that 104-76 and 352-57 represent secondary plasmacytomas induced in one of the tumor-bearing hosts. In this case, we could suppose that the presence of the original plasmacytoma was in some way responsible for the second. For example, the particles (viruses) found in plasmacytoma cells (5) could be infectious for nearby host cells or for attacking immune cells of the host, especially if the reaction of cells immune to the tumor were to cause activation and release of such viruses. The activation of murine leukemia viruses by graft-vs.-host reactions in vitro when correlated with the high incidence of leukemia in graft-vs.-host disease (22) supports the above possibility. Then, too, it seems significant in this context that a high and rapid incidence of leukemia can be induced in BALB/c mice with the Abelson virus (23).

Another way in which host immune cells could become neoplastic is through cell fusion with a plasmacytoma cell. In fact, cell fusion between two different Ig-producing plasmacytoma cells of BALB/c mice has been recently achieved in vitro, although in this particular case the Ig phenotypes of both parent cells continue to be produced by the hybrid cell line.[5] However, it is possible that according to the principle of allelic exclusion, only one of two allotypes would be produced in a hybrid cell for which the parent cells were each producing Ig allotypes of different mouse strains before cell fusion.

Consistent with either of the above mechanisms for the induction of a host tumor is the fact that 352-57 produces an F^{19} allotype that characterizes the IgG_1 of BALB/c mice in which MOPC-352 was always grown.

A second explanation for 104-76 and 352-57 is that these cell lines represent clonal variants of MOPC 104E and MOPC 352. Evidence for variable Ig production in some plasmacytomas is strong, beginning with the early work of Potter and Kuff (9), who showed that different nodules of a primary tumor could secrete the same L chain in combination with H-chains (HL_2 on HL) or as free L chains. With the adaptation of plasmacytomas to tissue culture, it was possible to analyze closely defective Ig-producing variants. And primarily from the elegant work of Scharff and co-workers (11, 24) and others (25), with cloned lines of plasmacytomas, the following is known: (*a*) that the generation of defective Ig-producing variants is spontaneous (11); and (*b*) that the frequency of variants is high (10^{-3}–10^{-4} per cell per generation) and peculiar to Ig production because the frequency of other variant cell traits such as resistance to drugs (puromycin, 6-thioguanine, 2-deoxyglucose, etc.) is less than 10^{-6} per cell per generation (24). On the basis of the preceding, any general instability of plasmacytoma cells is not likely to be the cause of altered Ig production. Rather, the appearance of defective Ig-producing cells is thought to reflect gene mutation or an unknown epigenetic mechanism (24) that could either result in altered gene transcription or altered assembly and secretion of Ig chains.

Unlike defective Ig-producing plasmacytomas, the two variants described in this paper produce intact Ig's that contain H or L chains that differ from those of

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[5] Margulies, D., W. M. Kehuel, and M. Scharff. Personal communication.

the "parent" lines. Results of a similar nature were recently reported by Preud'Homme et al. (26) for clonal variants of MPC-11 that produced altered IgG_{2b} H chains; these included H chains having serological, chemical, and assembly characteristics of IgG_{2a}-producing plasmacytomas. However, the MPC-11 variants were detected only after the cells were treated with the mutagens, ICR 191 or Melphalan. Mutagens were not used in our studies and we cannot easily attribute the variant Ig serotypes produced by cell lines 104-76 and 352-57 to mutation of Ig structural genes. Rather, we seem to be dealing with alterations in the regulation of Ig gene expression. Therefore, should gene mutation pertain, it may involve regulatory genes that control the expression of pre-existing Ig structural genes. For example, if different joining enzymes (or episomes) were responsible for the putative translocation of different sets of V and C genes, any genetic alteration of the specific joining enzymes active in a given plasmacytoma cell line could lead to an altered expression (or loss of expression) of the tumor Ig.

Irrespective of mechanism, altered transcription of Ig genes could serve as an explanation for the 352-57 variant. The parent cell line, MOPC 352, was derived from a BALB/c congenic mouse (CB-6) that was homozygous for a marker chromosome carrying the C57BL allotype genes.[4] However, we have strong evidence to suggest the presence of BALB/c allotype genes in CB mice, the products of which are normally undetectable by conventional assays (18, and our unpublished findings). Therefore, one could suppose that a chance expression of one of these hidden genes (F^{19}) in MOPC 352 occurred in a variant cell that had selective value in culture.

The difficulty in ascribing physiological meaning to the possibility of variable gene expression in the present work is twofold. First, plasmacytoma cells are highly-aneuploid and it is possible that anomalous chromosomal alterations could be responsible for the 104-76 and 352-57 variants. Secondly, we cannot be sure that the variants 104-76 and 352-57 reflect spontaneous, noninduced events as do for example the defective Ig-producing variants referred to earlier. Thus, it remains to be seen if variants like 104-76 or 352-57 can be selected from cloned lines of mouse plasmacytomas.

Summary

Murine plasmacytomas can be adapted to continuous in vitro culture by alternate passage between culture and animal. We have found that the kinetics of adaptation reflect a selection for the growth of variant plasmacytoma cells. The inclusion of an altered immunoglobulin phenotype in such variant cells could explain the Ig-producing variants that we observed in two of six transplantable lines of plasmacytomas that were adapted to culture. The first variant, an IgM-producing cell line (104-76), was adapted from a transplanted line of MOPC 104E that had stopped producing IgM with binding specificity for α1-3 Dextran. Unlike MOPC 104E, the IgM of 104-76 contains κ- instead of λ-light chains and probably contains an altered or different μ-heavy chain. A second variant (352-57) was found in an IgG_{2b}-producing tumor (MOPC 352) which was induced in a BALB/c mouse strain (CB-6) that carried Ig genes of the C57BL/Ka allotype. This cell line apparently switched from producing IgG_{2b} molecules of

the C57BL allotype (H^9) and of a known idiotype to IgG_1 molecules of the BALB/c allotype (F^{19}) without the idiotype marker. The propagation of a biclonal plasmacytoma from the time of original tumor induction does not appear as a likely explanation for these results. Rather, we seem to be dealing with plasmacytoma variants or with the possible induction of secondary tumors of host origin.

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