Proc. Natl. Acad. Sci. USA
Vol. 75, No. 3, pp. 1510–1514, March 1978
Immunology

Repertoire of antiviral antibodies expressed by somatic cell hybrids

(mouse hybridomas/influenza/parainfluenza)

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Contributed by Hilary Koprowski, December 22, 1977

ABSTRACT **Fusion between P3 × 63 Ag8 mouse myeloma cells and spleen cells from BALB/c mice immunized with influenza type A or B or parainfluenza type 1 virus generated reproducibly antiviral antibody-producing somatic cell hybrids (hybridomas). Eleven hybridomas derived from spleen cells of mice immunized with influenza type A virus were directed against the viral hemagglutinin, one reacted with a host component derived from chickens, and one expressed a specificity not further characterized. The hybridoma antibodies tended to be highly specific for the hemagglutinin of the immunizing virus and seemed to express the same repertoire of strain-specific antibody reactivities as splenic precursor B cells specific for the hemagglutinin of the immunizing virus strain. However, in contrast to splenic precursor B cells, they did not express any of the frequently occurring crossreactive anti-hemagglutinin specificities. Hybridomas producing crossreactive antibodies against hemagglutinin could be obtained if priming and boosting virus were heterologous.**

We have shown that somatic cell hybrids between mouse myeloma cells and spleen cells (hybridomas) from mice immunized with influenza virus produced antiviral antibodies (1). The production of antibody by hybridomas has obvious advantages over the classic way of raising antisera *in vivo*, since hybridomas may provide large amounts of antibody of a desired specificity and homogeneous binding characteristics. Such antibodies, which are specific for individual antigenic determinants, should greatly facilitate antigenic analyses of complex substances. However, in order for antibody-producing somatic cell hybrids to be generally useful, it should be possible to obtain hybridomas expressing any possible antibody specificity and to maintain them for long periods of time *in vitro* or *in vivo* without incurring changes in their capacity to produce antibody or in the antibody specificity.

In the present study, we investigated the general feasibility of somatic cell hybridization for antiviral antibody production using spleen cells from mice immunized with influenza type A or B or parainfluenza type 1 virus for cell fusion. Furthermore, we analyzed hybridoma and splenic antiinfluenza antibody clones by means of an anti-idiotypic antiserum raised against the anti-PR8 antibodies produced by the hybridoma PEG-1 and compared the repertoire of antibody-binding specificities expressed by hybridomas to that expressed by splenic precursor B cells of BALB/c mice.

MATERIALS AND METHODS

Virus. All virus strains were grown in the allantoic cavity of 10-day-old embryonated chicken eggs and were purified as described (2). The following type A influenza viruses were used to immunize mice: PR8 [A/PR/8/34 (HON1)], CAM [A/CAM/46 (H1N1)], and MEL [A/Melbourne/35 (HON1)]. These three strains and the following type A influenza viruses were used to analyze the antibody specificity in the radioimmunoassay: antigenic variants of PR8: V2, V3, and V6 (unpublished data); PR8-Mt. S (a PR8 strain maintained at Mt. Sinai Hospital); SW [A/Swine/33 (HswN1)]; WSN [A/WSN/33 (HON1)]; BH [A/BH/34 (HON1)]; HICK [A/Hickcox/40 (HON1)]; BEL [A/Bellamy/42 (HON1)]; WEISS [A/Weiss/45 (HON1)]; FM1 [A/FM/1/47 (H1N1)]; HK-X31 [A/Hong Kong/68 (N2H3)] (3); and recombinant viruses Eq-PR8 (Heq2N1) and JAP-BEL (H2N1). The type B influenza virus B/LEE and parainfluenza virus type 1, strain 6/94 (4), were used for immunization and assay.

Mice. Eight- to 10-week-old female BALB/c mice (Jackson Laboratories, Bar Harbor, ME, or Charles River, Wilmington, MA) were immunized by an intraperitoneal injection of 1250 hemagglutinating units of purifed virus in phosphate-buffered saline or 200 hemagglutinating units of high infectivity stock virus in allantoic fluid. Mice whose cells were to be used for fusion were boosted 4–6 months later by an intravenous injection of 200 hemagglutinating units of virus.

Production of Hybrid Cells. The preparation of BALB/c spleen cells, the origin and growth properties of the myeloma parent cells (P3 × 63 Ag8), and the method of fusing spleen cells and myeloma cells in the presence of polyethylene glycol (PEG) or inactivated Sendai virus (5) were as described (1). After fusion, cells were resuspended in hypoxanthine/aminopterin/thymidine selective medium (6) and seeded in 75-cm^2 Falcon flasks or in individual wells of Linbro plates FB16-24TC.

Cloning of Hybrid Cells. Initial hybrid cultures were cloned at limiting dilution in conditioned culture medium from P3 × 63 Ag8 myeloma cells or in soft agarose as described by Coffino and Scharff (7) but without a cellular feeder layer.

Splenic Fragment System. The production of monoclonal antibodies in the splenic fragment system has been described in detail (2).

Radioimmunoassay. The assay was done as described (1). The determination of the subunit specificity of antibodies was based on the interaction of antibodies with Eq-PR8, JAP-BEL, HK-X31, and B/Lee, which have different viral components in common with PR8 (8). The definition of the simplified reactivity type of antihemagglutinin (HA) antibodies was as follows (8): the amount of antibody in the test sample binding to homologous HA was defined as 100%; positive (+) and negative (−) interaction was given if ≥10% and <10%, respectively, of the antibody in the test sample bound to a heterologous HA.

Purification of PEG-1 Hybridoma Antibody and Preparation of Fab Fragment. Antisera produced by PEG-1 were purified from ascitic fluid by two cycles of low salt precipitation as described (1). The Fab fragment was prepared from purified PEG-1 antibodies by papain digestion and chromatographic

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Abbreviations: HA, hemagglutinin; PEG, polyethylene glycol 1000.

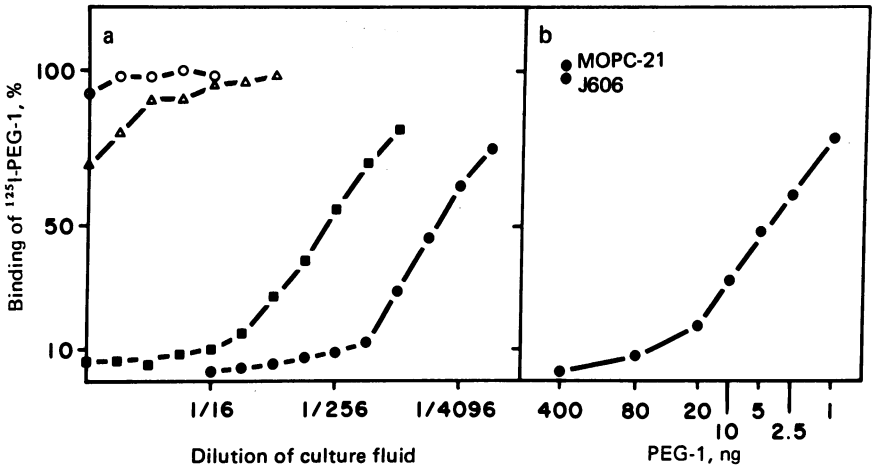

FIG. 1. PEG-1 idiotype assay. (*a*) Inhibition of binding of ^{125}I-PEG-1 by culture fluids of PEG-1 (●), H53/1 (■), H40/5 (▲), and P3 × 63 Ag8 (○). (*b*) Standardized curve of inhibition of purified PEG-1, quantitated on basis of A_{280}: 50% inhibition at 4 ng of PEG-1. Purified MOPC-21 (IgG1) and J606 (IgG3), both at 400 ng, show no inhibition.

separation on DEAE-cellulose as described by Spring and Nisonoff (9). The nature and purity of Fab-(PEG-1) was verified by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (10).

Production of Anti-PEG-1 Anti-Idiotype Antiserum. Two New Zealand white rabbits were injected subcutaneously at multiple sites with 0.4 mg of PEG-1 antibodies in complete adjuvant H37Ra (Difco Laboratories, Detroit, MI). The rabbits were boosted twice at intervals of 4–5 weeks with an intravenous injection of 0.1 mg of PEG-1 followed by an injection of 0.15 mg of Fab-(PEG-1). The rabbits were exsanguinated 14 days after the last injection. Antibodies directed against common Ig determinants were removed by precipitation of the serum with purified MOPC 21 (IgG1) and J606 (IgG3), the two isotypes secreted by the PEG-1 hybridoma (1). The adsorbed (anti-idiotype) serum was specific for idiotypic determinants of PEG-1 antibodies (Fig. 1*b*).

Idiotype Competition Assay. The procedure described by Cancro *et al.* (11) was followed. Briefly, a solid phase anti-idiotype immunoadsorbent was prepared by overnight passive adsorption at room temperature of 0.1 ml of anti-idiotype serum, diluted 1/10,000 in phosphate-buffered saline, to individual wells of polyvinyl plastic plates (Cooke Engineering, Alexandria, VA). Unabsorbed anti-idiotype serum was removed, 0.2 ml of 1% bovine serum albumin in phosphate-buffered saline was added per well, and the mixture was incubated for at least 1 hr at room temperature. The plates were rinsed and each well received 50 μl of competitor antibody (in phosphate-buffered saline containing 10% agamma horse serum) and 50 μl of purified ^{125}I-labeled PEG-1. The plates were incubated for 4 hr at room temperature and washed. The amount of ^{125}I-labeled PEG-1 (^{125}I-PEG-1) bound to the solid phase anti-idiotype of individual wells was determined in a gamma scintillation counter. The extent of inhibition of binding of ^{125}I-PEG-1 was standardized in each assay by samples containing known (on the basis of A280) quantities of unlabeled PEG-1 (Fig. 1*b*).

RESULTS

Production of Antiviral Antibody-Producing Hybrid Cell Cultures. Table 1 summarizes several fusion experiments performed with spleen cells of BALB/c mice immunized with influenza type A or B or parainfluenza virus. All cultures that

Table 1. Production of antiviral antibodies by hybrid cell cultures

Exp.	Spleen cells from BALB/c mice: Primed with	Spleen cells from BALB/c mice: Boosted with	Fusion in presence of	No. of spleen cells/culture ($\times 10^{-6}$)	Hybrid cultures*/total no. of cultures	Antiviral antibody-producing cultures/total no. of hybrid cultures
H35	A/PR8	A/PR8	Sendai	25–30†	0/5	0
H36	A/PR8	A/PR8	PEG	25–30†	5/5	5/5
H39	A/PR8	A/PR8	Sendai	25–30†	1/5	0/1
H40	A/PR8	A/PR8	PEG	25–30†	5/5	5/5
H52	A/CAM	A/PR8	Sendai	25–30†	0/5	0
H53	A/CAM	A/PR8	PEG	25–30†	5/5	4/4‡
H63	B/LEE	B/LEE	PEG	25–30†	10/10	8/8‡
H16	Parainfluenza 1	Parainfluenza 1	PEG	0.5§	32/32	24/32
H2	A/PR8	A/PR8	PEG	1.7§	90/90	87/87‡
H3	A/PR8	A/MEL	PEG	1.7§	91/96	67/88‡

* Number of cultures showing cell growth in selective hypoxanthine/aminopterin/thymidine medium.
† Cultures seeded in 75-cm^2 Falcon flasks.
‡ Some hybrid cultures were lost before determination of antiviral antibody production.
§ Cultures seeded in Linbro FB16-24TC plates.

Table 2. Specificity of antiinfluenza A antibodies produced by hybridoma clones

Hybridoma mass culture	No. of clones producing antibody/ total no. of clones isolated	Subunit specificity HA	NA*	ChHC†	Unknown
H40/2	10/11	10	0	0	0
H40/4	3/4	3	0	0	0
H40/5	7/7	7	0	0	0
H2/4B3	14/16	14	0	0	0
H2/4B5	7/8	7	0	0	0
H2/4C2	12/13	12	0	0	0
H2/6A5	3/10	3	0	0	0
H2/6C4	1/1	1	0	0	0
H3/4C4	3/17	3	0	0	0
H3/4C6	20/20	20	0	0	0
H53/1	14/26	0	0	0	7
H53/2	22/24	22	0	0	0
H53/5	9/48	3	0	1	0

* Neuraminidase.
† Chicken-derived host component.

showed growth of cells in hypoxanthine/aminopterin/thymidine selective medium were tested for production of antibodies binding in the radioimmunoassay with with the virus used for priming (and boosting) of the mice.

Antiviral antibody-producing hybrid cell cultures were obtained regardless of the virus used for immunization when fusion was performed in the presence of PEG. The high efficiency of PEG-induced fusion is clearly demonstrated by experiments H16, H2, and H3 in which 100, 100, and 95% hybrid cultures, respectively, were established although each individual culture comprised initially only 0.5×10^6, 1.7×10^6, and 1.7×10^6 spleen cells, respectively. In addition, 100 and 73% of the hybrid cultures produced antiviral antibodies. In contrast, the fusion experiments performed with inactivated Sendai virus resulted in only a single hybrid cell culture, which did not produce antiviral antibodies.

Specificity of Antibodies Secreted by Hybrid Cell Clones Derived from Spleen Cells with Primed Influenza Type A. Hybrid cell cultures were cloned in soft agar or at limiting dilution when the initial mass cultures were established in at least three 75-cm^2 Falcon flasks and showed vigorous cell growth. Table 2 shows that in many instances only a portion of the clones derived from a given hybrid culture produced detectable quantities of antiviral antibodies. The vast majority of these clones secreted antibodies with anti-HA specificity; one hybridoma produced antibodies against a host component derived from chicken and seven secreted antibodies of unknown specificities.

Table 4. Crossreactivity of strain-specific anti-HA(PR8) antibodies with antigenic variants of PR8 virus*

Hybridoma clone	PR8-parent	V2	V3	V6	PR8-Mt.S
H40/2-cl 14	≡ 6.64	5.46	0†	6.27	5.49
H40/5-cl 4	≡ 6.64	6.02	0	6.46	5.83
H2/4B3-cl 13	≡ 6.64	0	0	6.00	6.00
H2/4B5-cl 4	≡ 6.64	6.04	6.02	6.23	0
H2/6A5-cl 4	≡ 6.64	5.00	0	5.46	3.46
H2/6C4-cl 13	≡ 6.64	6.17	5.78	6.02	6.41

* Values given are the amount ($\log_2$) of hybridoma antibody binding in the radioimmunoassay to PR8 variants. The computation of the entries is explained in *Results*.
† 0, ≤1/100 of antibody binding to priming virus reacts in the radioimmunoassay with heterologous virus.

The various anti-HA hybridoma antibodies were further tested for their crossreaction with a panel of nine heterologous influenza viruses of A0 and A1 subtypes (Table 3) and with antigenic variants of PR8 (Table 4). The extent of crossreaction of hybridoma antibodies with the various viruses is expressed in $\log_2$ and is normalized so that the interaction with the virus used for priming (PR8 or CAM) ≡ $\log_2$ (100). The average standard deviation of these normalized binding values is 0.36. This was determined with 10 antibodies in 4 to 7 repeated assays and comprised 40 crossreactions of different extents, ranging from 1 to 7.0. Thus, given a 99% significance limit of ±0.97 for individual binding values, a difference of more than 1.94 between two binding values was regarded as highly significant.

Since all anti-HA(PR8) hybrid clones derived from the same mass culture exhibited identical types of crossreaction, the results of these analyses refer to only a single clone from each mass culture. It is evident from Table 3 that all crossreactive hybridomas differed from each other with regard to the extent of crossreaction. For instance, H3/4C4-cl 9 and H3/4C6-cl 16 differ significantly in their interaction with MEL. Similarly, Table 4 shows that with the possible exception of H40/2-cl 14 and H40/5-cl 4, the HA(PR8)-specific hybridomas differed from each other in their reactions with antigenic variants of

Table 3. Crossreactivity of hybridoma antibodies with various strains of influenza A virus*

Hybridoma clone	(HswN1) SW	(H0N1) PR8	WSN	BH	MEL	HICK	BEL	WEISS	(H1N1) CAM	FM1
H40/2-cl 14	0†	≡ 6.64	0	0	0	0	0	0	0	0
H40/4-cl 1	2.00	≡ 6.64	0	0	0	0	0	0	0	0
H40/5-cl 4	0	≡ 6.64	0	0	0	0	0	0	0	0
H2/4 B3-cl 13	0	≡ 6.64	0	0	0	0	0	0	0	0
H2/4 B5-cl 4	0	≡ 6.64	0	0	0	0	0	0	0	0
H2/4 C2-cl 2	0	≡ 6.64	2.81	2.81	0	2.58	2.58	1.00	1.58	2.32
H2/6 A5-cl 4	0	≡ 6.64	0	0	0	0	0	0	0	0
H2/6 C4-cl 13	0	≡ 6.64	0	0	0	0	0	0	0	0
H3/4 C4-cl 9	3.97	≡ 6.64	<2	<2	5.17	3.38	<2	<2	<2	<2
H3/4 C6-cl 16	2.32	≡ 6.64	0	1.58	2.00	3.32	0	2.81	3.58	3.32
H53/2-Ag-cl 22	0	3.46	0	0	0	7.15	0	0	≡ 6.64	4.00
H53/5-LD-cl 8	1.00	5.58	5.09	4.86	0	6.74	1.00	6.27	≡ 6.64	6.86

* Values given are amount ($\log_2$) of hybridoma antibody binding to viral immunoadsorbents in the radioimmunoassay. The computation of the entries is explained in *Results*.
† 0, ≤1/100 of antibody binding to priming virus reacts in the radioimmunoassay with heterologous virus.

Table 5. Reactivity type of hybridoma and splenic anti-HA(PR8) antibodies

Type of antibody*	Reactivity type†					No. of antibody clones	
	PR8	V2	V3	V6	Mt.S	Splenic	Hybridoma‡
PR8-specific	+	+	+	+	+	7	2 (H2/4C2-cl 2, H2/6C4-cl 13)
	+	+	+	+	−	3	1 (H2/4B5-cl 4)
	+	+	−	+	+	4	4 (H40/2-cl 14, H40/4-cl 1, H40/5-cl 4, H2/6A5-cl 4)
	+	−	+	+	+	3	0
	+	−	−	+	+	1	1 (H2/4B3-cl 13)
	+	−	−	−	+	0	1 (PEG-1, cl 6)
Crossreactive						78	0

* HA(PR8)-specific: antibodies that did not exhibit a crossreaction with a heterologous virus exceeding 10% ($\log_2 = 3.32$) of the homologous reaction. Crossreactive: anti-HA antibodies that reacted (>10% of homologous interaction) with one or several of the heterologous influenza viruses shown in Table 3.

† Reactivity type of an antibody is defined in *Materials and Methods*.

‡ Hybridomas exhibiting the indicated reactivity type are given in parentheses.

PR8. It is also worth noting that hybridomas derived from spleen cells of mice primed and boosted with PR8 tended to be specific for the HA of PR8 (Table 3, H40 and H2 clones; see also Table 1). In contrast, all hybridomas obtained by fusion of spleen cells from mice primed with PR8 and boosted with MEL (H3) or primed with CAM and boosted with PR8 (H53) were crossreactive. Furthermore, the hybridomas obtained by heterologous boosting reacted with both priming and boosting viruses, although reactions were better with the former.

Comparison of Hybridoma and Monoclonal Splenic Antibodies with Regard to Specificity of Combining Site and Idiotype. In order to determine whether the observed hybridoma antibody specificities represent frequently occurring specificities expressed by the secondary anti-PR8 precursor cell pool of the BALB/c mouse, the specificities of hybridoma antibodies and monoclonal splenic antibodies were compared (Table 5). The splenic antibody clones were derived from the adoptive transfer of five individual BALB/c spleen cell preparations, obtained 4–6 months after animals were primed with PR8, into lethally irradiated syngeneic recipient mice. Splenic fragments of the recipients were stimulated *in vitro* with the homologous virus. This comparison is, therefore, only relevant for the H2 and H40 hybridomas (see Table 1) and for PEG-1, a hybridoma that produces anti-HA(PR8) antibody and has been described in detail (1). In order to facilitate the comparison, antibodies are grouped in Table 5 on the basis of simplified reactivity type.

Table 5 shows that 81% of the splenic monoclonal anti-PR8 antibodies are crossreactive, i.e., react with HA(PR8) and one or several heterologous HA (see legend of Table 5). This is in marked contrast to the predominant specificity of the hybridoma antibodies for the homologous HA. On the other hand, hybridoma antibodies seemed to express a repertoire of PR8-specific reactivity types similar to those of splenic antibody clones.

Table 5 also shows that none of the splenic antibody clones expressed the reactivity type of PEG-1. Thus, in order to verify the low frequency of antibodies with the reactivity type of PEG-1, all 96 splenic antibody clones were tested in the idiotype assay for the expression of PEG-1 idiotypic determinants. The sensitivity of the assay for the PEG-1 idiotype is shown in Fig. 1*b*. None of the splenic antibody clones tested at concentrations between 5 and 10 ng/50 μl inhibited the binding of ^{125}I-PEG-1 by greater than 20%, thus confirming the absence of the PEG-1 idiotype among these 96 normal splenic antibody clones. The same result was obtained with a mouse anti-PEG-1 idiotypic antiserum. Similarly, none of the hybridoma clones included in Table 5 expressed the PEG-1 idiotype. In contrast, of ten hybridoma mass cultures tested, one (H53/1) inhibited the binding of ^{125}I-PEG-1 up to 94–95% and, therefore, appeared to contain antibodies expressing idiotypic determinants highly crossreactive to but probably not identical with the PEG-1 idiotype (Fig. 1*a*). These crossreactive idiotypic determinants were present in H53/1 mass culture fluid at roughly 10% lower concentration than in PEG-1 culture fluid. However, none of the seven randomly selected hybridoma clones derived from H53/1 expressed an idiotype crossreactive with PEG-1.

Table 6. Functional stability of hybridoma PEG-1

Immunoadsorbent	Reactivity ($\log_2$) of PEG-1, cl 6 passaged: *In vivo*:* Generation 4	8	11	*In vitro*:† Passage no. ×1	×20	×28
PR8	≡ 6.64	≡ 6.64	≡ 6.64	≡ 6.64	≡ 6.64	≡ 6.64
Mt.S	5.98	6.19	6.39	6.04	6.39	6.04
V6	1.63	2.20	2.14	2.59	2.56	2.59

Antibody concentration, based on idiotype assay, was 2.3 mg/ml (range 1.3–5.6) *in vivo* and 0.079 mg/ml (range 0.063–0.102) *in vitro*.

* Passage of PEG-1 as ascites as described in text.

† Approximate passage number of PEG-1 culture *in vitro*: ×1, initial culture fluid; ×20, culture fluid 51 days later, ×28, pooled culture fluid 62–70 days later.

Maintenance of Hybridomas. In order to obtain some information about the stability of hybridomas, clone 6 of hybridoma PEG-1 was passaged consecutively in ascitic form (2×10^5 cells intraperitoneally per passage) and *in vitro* (1:4–1:6 dilution of the culture every 2–3 days). Table 6 shows that in the course of *in vivo* and *in vitro* passages, PEG-1 retained its capacity to produce antiviral antibodies. Furthermore, the reactivity type of the antibody did not change signficantly, as seen by its crossreaction with PR8-Mt.S and the PR8 variant V6.

DISCUSSION

Fusion in the presence of PEG between P3 × 63 Ag8 myeloma cells and spleen cells from BALB/c mice immunized with influenza type A or B or parainfluenza type 1 virus generates reproducibly antiviral antibody-producing hybrid cells. Furthermore, as demonstrated by clone 6 of the anti-HA(PR8) hybridoma PEG-1, cloned hybrid cell lines seem to be stable with regard to their antibody-producing capacity and the specificity of the secreted antibodies. Although individual hybridomas may vary with regard to their stability (12) and may require recloning after a number of passages, somatic cell hybridization seems to represent a generally applicable methodology for the production of large quantities of defined antibodies against viruses.

Antigenically stimulated B cells seem to have a selective advantage over resting B cells in the process of hybridization with meyloma cells. For instance, 100% of the cultures in fusion experiment H2 (Table 1) gave rise to antiviral antibody-producing hybrids despite the fact that each culture comprised initially only 1.7×10^6 spleen cells. Furthermore, 77% of the clones derived from five of these cultures produced antiviral

antibodies. This is surprising since the absolute frequency of secondary HA(PR8)-committed precursor B cells is roughly 1/5000 splenic B cells (2) and HA(PR8)-specific precursors (the splenic parent cells of the five antiviral antibody-producing hybrid cultures analyzed so far) represent approximately 20% of the total HA (PR8)-committed precursor cell pool, i.e., 1/25,000 splenic B cells (Table 5). Thus, although the HA(PR8)-specific precursors may have undergone several cycles of division in the 3 days after antigenic boost *in vivo*, they must still have represented a considerable minority of the total B cells present at the time of fusion. These observations are in agreement with the previous findings of Köhler and Milstein (12) and Galfre *et al.* (13), who observed a high frequency of hybrids producing antibodies against the antigen used for immunization, i.e., sheep erythrocytes and rat lymphoid cells, respectively.

Analysis of 205 hybrid clones derived from 13 individual hybrid cultures revealed a surprisingly low cellular heterogeneity of hybrid mass cultures which was in general shown only by the presence or absence of antiviral antibody production by individual hybrid clones. On the other hand, all antiviral antibody-producing clones derived from the same culture appeared to represent, as judged by the identical reactivity of the secreted antibodies, identical hybrids. This observation was independent of the number of spleen cells used per culture and therefore of the presumed number of fusion events originally occurring per culture. Thus, it seems that hybrid cultures are polyclonal at first but are usually rapidly overgrown by one or very few fast growing hybrids. Support for this assumption also comes from the observation that individual hybrid cultures of experiments H2 and H3, which comprised the same number of spleen cells and were kept under identical conditions, reached comparable cell densities at quite different times ranging from 8 to 20 days after fusion. Consequently, in order to generate a large variety of hybridomas a minimum number of spleen cells per initial hybrid culture should be used.

In order to be of general usefulness, especially with regard to antigenic analyses, somatic cell hybrids should be able to express the complete repertoire of antibody specificities. This point was further investigated by comparing the specificities expressed by anti-HA(PR8) hybridomas with those expressed by monoclonal antibodies produced in the splenic fragment culture system (2, 8). Analysis indicated that the hybridoma antibodies did express the same repertoire of strain-specific reactivity type as splenic antibodies. However, they obviously lacked the various crossreactive reactivity types which comprise roughly 80% of the splenic anti-HA(PR8) response. The reason for this discrepancy between the two systems is not known. It may be due to the different virus dose used for antigenic boost of the precursor cells *in vivo* and *in vitro*. It could also result from the possibility that a large fraction of hybridoma antibodies are monovalent with regard to their antiviral antibody binding activity and that many low-affinity crossreactions require bivalent antigen–antibody interactions to be detectable in the radioimmunoassay. It is also conceivable that specific and crossreactive precursors differ in their kinetics of differentiation after homologous antigenic boost and that only a certain stage of differentiation provides the B cell with the selective advantage in the process of fusion. However, one can exclude the possibility that certain subpopulations of the anti-HA(PR8) B cell repertoire are not at all susceptible to hybridization with the myeloma cell P3, since crossreactive hybrids were obtained when priming and boosting viruses were heterologous. The results reported here suggest that by using the right experimental conditions, the whole repertoire of anti-HA(PR8) antibody specificities may be obtained from antibody-producing hybrid clones.

The excellent technical assistance of Mrs. Maureen Carey and Roseann Mazzola is gratefully acknowledged. This work was supported, in part, by National Institutes of Health Grants NS-11036, AI-13989, GM-02051, GM-20700, CA-16685, and CA-20741, by the American Cancer Society (VC220), by The National Foundation of the March of Dimes (1-77-42), and by the National Multiple Sclerosis Society. C.M.C. is a recipient of Research Career Development Award CA-00143 from the National Cancer Institute.

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