

## Methods for the Enrichment of Desired B-Cell Populations Before Anti-Cauliflower Mosaic Virus Hybridoma Formation

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### ABSTRACT

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Relative to a standard procedure and adjusted to uniform population sizes, immune complex masking, solid-phase immunoabsorption, and complement-mediated cytotoxicity used before murine hybridoma formation reduced by 49, 80, and 77%, respectively, the undesired anti-healthy Chinese cabbage (HCC) clones produced during studies on the production of anti-cauliflower mosaic virus (CaMV) monoclonal antibodies by hybridoma formation made from spleen cells from mice immunized with partially purified preparations of CaMV from infected Chinese cabbage leaves. These reductions led to 49, 72, and 54% increases,

respectively, in the number of anti-CaMV clones found during screening. Analysis of the murine splenocyte populations by plaque-forming cell assay showed that these procedures resulted in reductions of anti-HCC B-cell populations of 49% for immune complex masking, 80% for solid-phase immunoabsorption, and 77% for complement-mediated cytotoxicity, compared to levels found by using a standard fusion procedure, thus enriching the level of desired anti-CaMV B cells in the resulting suspensions.

The advent of monoclonal antibody technology has made it possible to develop antibodies for certain plant viruses where previous techniques were unsatisfactory (3,9,10,17,21,24). As the technique continues to be refined for plant virology and becomes more broadly used for taxonomic and epidemiological, as well as diagnostic purposes, the need for even greater specificity becomes apparent. In the preparation of monoclonal antibodies against certain plant viral antigens, the enhancement of B-cell numbers reactive against those desired antigens would be very useful for viruses that occur in low titer or for which antisera of any kind are lacking.

A desirable fusion involves combination of a murine myeloma cell with a specific idiotype B cell that is present in a large mixed population of B cells (1,20,25). Several techniques have been employed to divide the various B and T cell populations and subpopulations into groups by using immunochemical or physical properties (2,4,5,18-20,27). Other techniques have been used to mask possible antigens, thus reducing the presence of an undesired resulting population of B cells (14).

While working on the serological relationships within the caulimovirus group (13) and trying to develop hybridomas to blueberry red ringspot virus (11) and cauliflower mosaic virus (CaMV) (13), we encountered a high rate of anti-healthy plant antigen clones (about 40%/fusion) while the number of viral antigen-specific clones was unsatisfactorily low. Polyclonal sera collected from hyperimmune mice at the time of spleen removal had high virus-specific titers and low anti-healthy plant sap titers (11). It became apparent that the selective enrichment of specific B-cell populations would be a useful supplement to existing purification procedures. By selectively modifying this B-cell population, a greater number of resulting antiviral hybridomas might be obtained.

The CaMV system was chosen as a model because its purification and serology have been extensively studied (13,23). We examined three procedures, analogous to the B and T cell studies cited previously, of selectively enriching the populations of

desired B cells in mice: 1) B-cell reduction by immune complex masking; 2) B-cell reduction by solid-phase immunoabsorption; and 3) complement-mediated B-cell lysis. These three procedures (hereafter referred to in brief as masking, adsorption, and lysis procedures) were used for the selective enrichment of mouse B-cell populations to increase the number of CaMV-specific antibody-producing hybridomas. To our knowledge, this is the first time these methods have been applied to plant viral-hybridoma systems. An abstract concerning this work has appeared (8).

### MATERIALS AND METHODS

**Preparation of anti-healthy antibody.** Four BALB/c mice were immunized with four separate injections of purified healthy Chinese cabbage (HCC) (*Brassica pekinensis* (Lour.) Rupr.) prepared by the CaMV purification procedure of Pirone et al (23). This included two cycles of 10-40% rate-zonal sucrose density gradient ultracentrifugation, subsequent fractionation, and also ultracentrifugation of the CaMV-comparable HCC fractions.

Ascites tumors were induced in the mice by repeated injections of 1:1 suspensions of HCC antigen in Freund's complete adjuvant, following the method of Scott et al (26). If tumors failed to develop, P3X63AG8.653 (P3X) murine myeloma cells, kindly supplied by E. L. Halk (Agrigenetics Corp., Madison, WI), were injected intraperitoneally.

**Preparation of anti-healthy idiotype antibody.** Ascitic fluid was collected when the mouse tumor had developed sufficiently, and anti-HCC immunoglobulins were obtained from the ascitic fluid by passage through a CM AffiGel Blue column (Bio-Rad Labs, Richmond, CA). The concentration was determined using the extinction coefficient of  $1.4 \text{ mg ml}^{-1} \text{ cm}^{-1}$  at 280 nm, and 0.750 mg/ml aliquots were made and stored frozen at  $-74 \text{ C}$ . These aliquots were mixed 1:1 with Freund's complete adjuvant and used to immunize a New Zealand White rabbit doe by intradermal injections (28) on a biweekly basis for 2.5 mo. The final booster injection at 2 mo 21 days was intramuscular. The rabbit was bled 14 days after the booster injection. The rabbit (anti-HCC idiotype immunoglobulin) antiserum was repeatedly adsorbed (15) on a Protein A-Sepharose 4B column (Pharmacia Fine Chemicals, Piscataway, NJ) containing a pool of bound mouse normal

immunoglobulin until all detectable reactivity was removed, as evaluated by direct double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (6). The resulting immunoglobulins were concentrated by saturated ammonium sulphate precipitation and adjusted to 1 mg of immunoglobulin per milliliter.

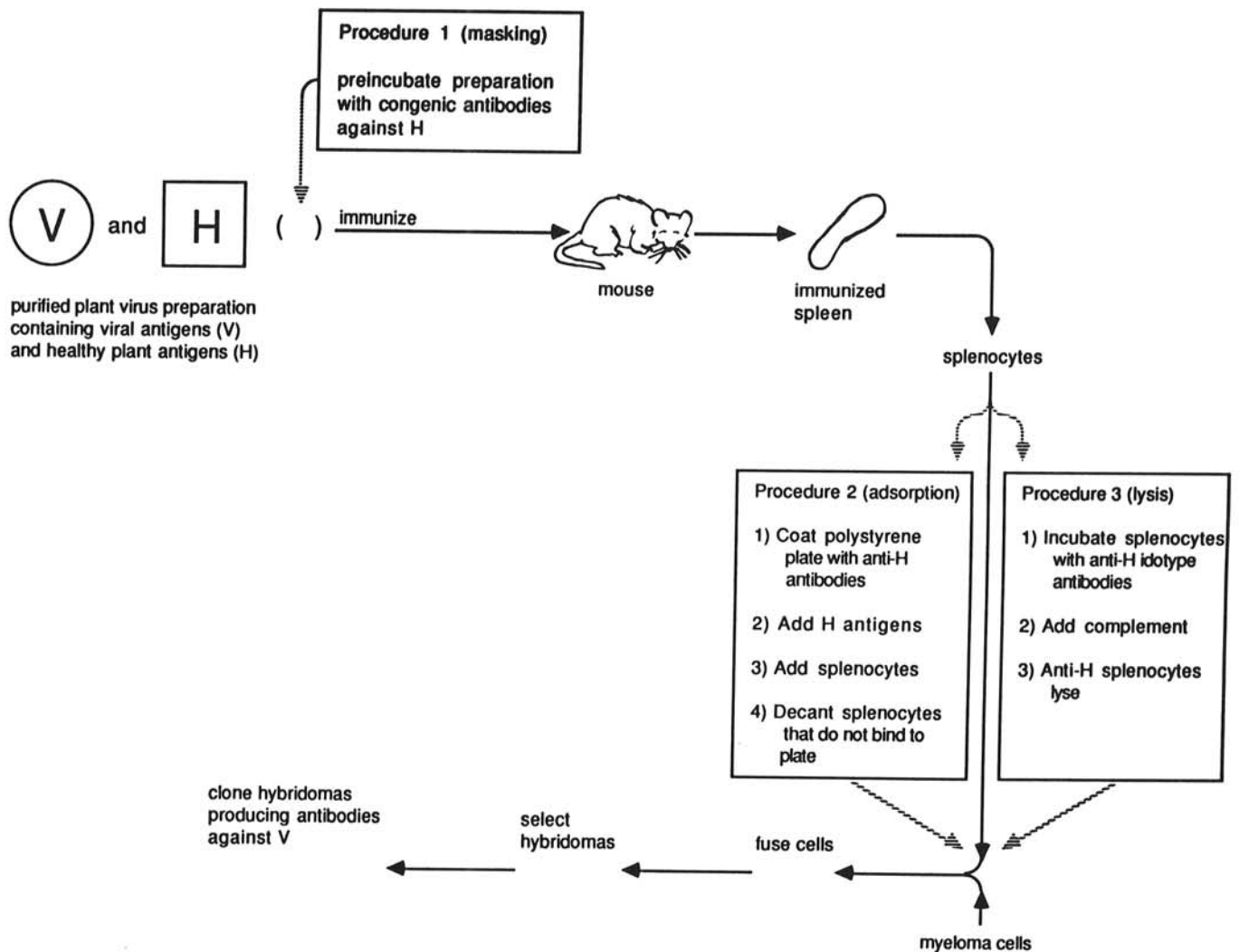
**Fusion procedure.** Eight BALB/c mice per experiment received three intraperitoneal injections at biweekly intervals with either 500  $\mu$ g of partially purified CaMV (23) from Chinese cabbage (hereafter referred to as CaMV-CC) in 100  $\mu$ l of 0.1 M phosphate-buffered saline, pH 7.2 (PBS), or emulsified 1:1 in Freund's complete adjuvant. The third injection was followed 20 days later by a fourth injection of 150  $\mu$ g of CaMV-CC in 100  $\mu$ l of PBS. Three days after the fourth injection spleens were harvested and pooled.

The pooled spleens were portioned into three aliquots, of which two were used for two of the three selective B-cell enrichment procedures to be described. The third aliquot was used directly for fusion with P3X murine myeloma cells using 35% polyethylene glycol (MW 1500) (BDH Chemicals, Ltd., Poole, England) according to the standard method of Köhler and Milstein (17). Four BALB/c mice were injected with CaMV-CC preincubated with congeneric antibodies against HCC for immune complex masking (see below), the first of the three selective B-cell enrichment procedures that we tested. A flow chart (Fig. 1) shows the standard fusion procedure schematically and the sites of each of the three selective B-cell enrichment procedures.

*Enrichment procedure 1: B cell reduction by immune-complex masking (masking procedure).* The maximum titer of mouse anti-HCC immunoglobulin needed to completely inhibit DAS-ELISA response from HCC leaf sap purified according to the methods used for CaMV purification (23) was determined. This involved coating ELISA plates (Immulon 2, Dynatech Corp., Chantilly, VA) with mouse anti-HCC immunoglobulin at a constant concentration in ELISA coating buffer ( $\text{Na}_2\text{CO}_3$  1.59 g,  $\text{NaHCO}_3$  2.93 g,  $\text{NaN}_3$  0.2 g in 1 L of distilled water adjusted to pH 9.6 with HCl as needed). Purified HCC leaf sap was incubated overnight at 4 C with mouse anti-HCC immunoglobulin at concentrations that ranged from 2.0 mg/ml to 0.01  $\mu$ g/ml. After low-speed centrifugation to remove sediment, these samples were added to rinsed ELISA plates, four replicate wells per concentration level, followed by a 4-hr incubation at room temperature. Mouse anti-HCC immunoglobulin conjugated with alkaline phosphatase (Sigma Chemical Co., St. Louis, MO) was added to the plate. Absorbance was measured at 405 nm (6).

The maximum antigen binding capacity of mouse anti-HCC immunoglobulin was determined by standard graphic methods, and a fivefold excess was added to CaMV-CC purified preparations to yield HCC immune complexes with excess antibody. These preparations were then used to immunize four congenic mice (i.e., also BALB/c mice) as described earlier, and the fusion process was performed using conventional methods (17) as shown in Figure 1.

*Enrichment procedure 2: B-cell reduction by solid-phase*



**Fig. 1.** Schematic representation of the murine hybridoma fusion process indicating where three selective B-cell enrichment procedures: Procedure 1, immune complex masking; Procedure 2, B-cell adsorption; and Procedure 3, B-cell complement-mediated lysis, are incorporated. The standard fusion process omits all three B-cell enrichment procedures.

*immunoadsorption (adsorption procedure)*. A 10-ml volume of sterile mouse anti-HCC serum diluted 1:10 in ELISA coating buffer was added to a sterile polystyrene petri plate (Fisher Scientific Co., Pittsburgh, PA) and the plate incubated at 4 C overnight. The plate was drained and rinsed twice with sterile PBS-Tween 20, pH 7.2. Healthy Chinese cabbage leaves were homogenized (Polytron, Brinkmann Instruments, Westbury, NY) in PBS-Tween 20 (1:10, w:v), expressed through cheesecloth, and the supernatant fluid from low-speed centrifugation (8,000 g for 10 min) was filter-sterilized by passing it through a sterilization filter unit (0.2  $\mu$  pore size) (Nalge Co., Rochester, NY). The resulting sterile HCC sap was added to the plate, which was incubated and rinsed. An anti-CaMV-CC mouse splenocyte suspension was poured into the plate, which was then incubated undisturbed for 15 min at room temperature. The plate was agitated gently to resuspend anti-CaMV and other splenocytes not bound to the plate. The resulting suspension was gently drawn off so as to leave the anti-HCC splenocytes adhering to the plate. The fusion process was then completed as shown in Figure 1.

*Enrichment procedure 3: Complement-mediated B-cell lysis (lysis procedure)*. An anti-CaMV-CC splenocyte suspension was washed with Dulbecco Modified Eagle's Medium (DMEM) and then resuspended in 10 ml of DMEM to which had been added 200  $\mu$ g of rabbit anti-HCC-idiotype immunoglobulin. The mixture was incubated 15 min at 37 C, centrifuged (125 g for 10 min), and the pellet resuspended in 10 ml of agarose-adsorbed sterile guinea pig serum (as a source of complement components) (JEM Research Inc., Kensington, MD) diluted 1:10 in PBS. This mixture was incubated 45 min at 37 C, then washed with DMEM, followed by a 15-min incubation in 350 units/ml of trypsin (Sigma) with 1 unit/ml of DNase (Sigma) to assist in the degradation of the products of complement lysis, after the methods of Hirata (12) and Klein and Perlmann (16). Cells were washed and the entire process repeated. Rabbit normal globulin used in the same manner served as one internal control, and a no-rabbit-globulin, no-complement system served as a second internal control to monitor handling and any nonspecific reactivity associated with the procedure. The final suspension was washed with DMEM and the fusion process completed, as shown in Figure 1.

**Hybridoma selection.** Fused cells from each fusion experiment were seeded directly into five 96-well plates (Nunc, Vanguard International Inc., Neptune, NJ). HAT selective medium (22), which contained DMEM supplemented with hypoxanthine, aminopterin, thymidine, and 15% fetal calf serum with mouse thymocytes as feeder cells, was added to the plate wells. After 10 days, wells with microscopically visible colonies were screened for specific antibody production using the triple antibody sandwich ELISA described by Martin and Stace-Smith (21) with the following modification: Rabbit anti-CaMV immunoglobulin was used to coat Immulon 2 ELISA plates (100  $\mu$ l/well = 0.1  $\mu$ g) and incubated overnight at 4 C. Plates were rinsed and 100  $\mu$ l of CaMV-infected or crude HCC leaf sap (1:10, w:v) in PBS-Tween 20 with 0.2% bovine serum albumin, 2% polyvinyl pyrrolidone (MW 10,000) added, and the plates incubated 4 hr at room temperature. After rinsing, 50  $\mu$ l of hybridoma culture supernatant fluid plus 50  $\mu$ l of PBS Tween 20 were added to each well for 4 hr at room temperature. Rabbit anti-mouse immunoglobulin (Organon Teknika-Cappell Co., Malvern, PA) conjugated with alkaline phosphatase was added at 1:1,000 in PBS-Tween 20 for 4 hr, and finally, after suitable rinsing, *p*-nitrophenylphosphate at 1 mg/ml was added in diethanolamine, pH 9.6. The culture fluid control consisted of polyclonal mouse anti-CaMV. Buffer-filled wells were included in each plate.

Positive wells were selected by applying a threshold value (defined as mean absorbance for four wells/plate with HCC sap plus four standard deviations of that mean after 45 min of incubation). There were two replicates per tested hybridoma well, and the screening was repeated three times on days 10, 14, and 18 following the fusion. Hybridomas retained after screening were obtained as monoclones by use of standard limiting dilution techniques (17) and subsequently increased.

**Methods used to calculate relative efficiencies of hybridomas**

**produced by use of three B-cell enrichment procedures.** Efficiencies of the enrichment procedures were determined by comparing the number of hybridomas that produced CaMV antibodies by the standard technique with the number produced by the three enrichment procedures (masking, adsorption, and lysis). Additional comparisons were made with internal controls that paralleled each method but omitted one or more of the reactants during the incubation period.

These comparisons required adjustment for variation in the total number of hybridomas produced by use of each of the three enrichment procedures in comparison to that produced by the standard procedure. This adjustment was accomplished by use of equation 1:  $x = 100 [(ac/b) - d]/d$ , where  $x$  is the percentage increase or decrease over standard procedure of desired hybridomas using a given enrichment procedure;  $a$  is the observed number of desired hybridomas using that enrichment procedure;  $b$  is the total number of hybridomas produced by that enrichment procedure;  $c$  is the total number of hybridomas produced by the standard procedure; and  $d$  is the number of desired hybridomas produced by the standard procedure. An example will be given in the Results section, using the adsorption procedure.

**Relative efficiencies of enrichment procedures for producing desired B cells as measured by plaque assay.** An additional 12 mice were used to study the effects of the masking, adsorption, and lysis procedures on the splenocyte population. This direct examination employed a plaque-forming cell (PFC) assay (7).

For the masking procedure, splenocyte suspensions from six BALB/c mice, two immunized with a purified CaMV-CC preparation in the presence of an excess of congenic anti-HCC antibody prepared as described earlier, two immunized with a purified CaMV-CC preparation but with no congenic anti-HCC antibody, and two immunized with 0.1 M PBS, pH 7.2, were prepared by splenectomy and maceration of the spleen capsule. The cells were centrifuged, then mixed in suspension with sheep red blood cells that had been sensitized by coupling HCC to them by 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-*p*-toluene sulfonate (Sigma) conjugation (14). This suspension was plated out in the presence of complement in Cunningham chambers (7) and evaluated for the number of plaques that developed.

For PFC assay of the adsorption procedure, splenocyte suspensions from three mice were divided into four equal portions. One portion was incubated on HCC-sensitized plates, the second on plates coated with mouse normal serum, the third on noncoated plates, and the fourth was not incubated. Once incubation had been completed, the cells were gently agitated and the resulting suspension transferred to centrifuge tubes. The cells were pelleted and then resuspended with HCC-conjugated sheep red blood cells. This suspension was plated out (7) and evaluated for the number of plaques that developed.

For PFC assay of the lysis procedure, splenocyte suspensions from three mice were pooled, treated by complement as described earlier, and divided into four equal portions. The standard lysis procedure was used for one portion in the presence of rabbit anti-idiotype antibodies. The second portion received the lysis procedure in the presence of rabbit normal globulin. The third received only complement, and the fourth was untreated (i.e., no complement and no rabbit globulin). These four splenocyte suspensions were then plated and evaluated as described above.

## RESULTS

**Preliminary evaluation of the enrichment procedures.** A system using rabbit normal globulin as the test antigen was used in preliminary studies to evaluate the three enrichment procedures. All hybridomas produced against rabbit normal globulin were considered undesirable in the study, so the absence of anti-rabbit-normal-globulin hybridomas after application of masking, adsorption, and lysis procedures was desirable. Incorporation of each of these procedures resulted in marked reduction of hybridomas producing antibodies against rabbit normal globulin (data not shown).



A PFC assay of anti-rabbit normal globulin splenocyte suspensions after the use of the masking, adsorption, and lysis procedures showed splenocyte reductions of 53, 83, and 64%, respectively, compared to nontreated suspensions (data not shown).

Formation of hybridomas from mice immunized with rabbit normal globulin paralleled these earlier analyses, with 65% reduction of anti-rabbit normal globulin-specific hybridomas after the masking procedure, 83% reduction after the adsorption

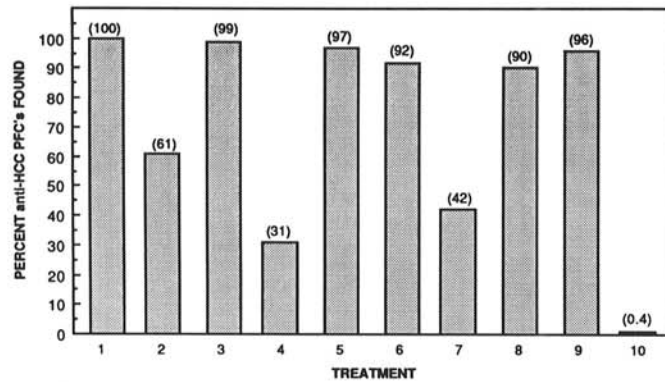


Fig. 2. Bar chart of the percent of undesired (anti-HCC) plaque-forming B cells (PFCs) found following various procedures for selective B-cell reduction when compared to a standard procedure. Treatments are: 1) standard procedure; 2) B cells from mouse spleens immunized with a purified preparation of CaMV-CC in the presence of excess congenic anti-HCC antibodies to give immune complex masking; 3) treatment 2 without anti-HCC antibodies; 4) solid-phase immunoadsorption; 5) treatment 4 with mouse normal globulin attached to the solid phase; 6) treatment 4 with no globulin attached to the solid phase; 7) complement-mediated B-cell cytotoxicity; 8) complement plus rabbit normal globulin; 9) no rabbit globulin, no complement; and 10) immunization with 0.1 M PBS, pH 7.2 only. CaMV-CC = cauliflower mosaic virus purified from Chinese cabbage; HCC = healthy Chinese cabbage antigens; PBS = phosphate-buffered saline.

procedure, and 70% reduction after use of the lysis procedure when compared to the standard fusion procedure (data not shown).

**Plaque-forming cell assays of anti-CaMV-CC splenocytes.** The PFC assay for splenocyte suspensions from CaMV-CC-immunized mice subjected to the masking procedure had a 39% reduction in anti-HCC-specific B cells (Fig. 2, treatment 2) when compared to splenocyte suspensions derived from mice immunized with CaMV-CC by the standard procedure. The internal control showed a 1% reduction (Fig. 2, treatment 3).

A similar assay of anti-CaMV-CC splenocytes subjected to the adsorption procedure (Fig. 2, treatment 4) showed a 69% reduction in anti-HCC PFCs compared to the standard procedure, while the internal controls, solid phase blocked by mouse normal globulin (Fig. 2, treatment 5) and noncoated plates (Fig. 2, treatment 6), had reductions of 3 and 8%, respectively.

PFC assay of anti-HCC-specific B cells subjected to the lysis procedure had a 58% reduction of these B cells when compared to the standard procedure (Fig. 2, treatment 7). The PFC assay of the internal controls (Fig. 2, treatments 8 and 9) showed 10 and 4% reductions in total PFC counts for complement, no-rabbit globulin and for no complement, no-rabbit-globulin, respectively.

**Direct detection of anti-CaMV hybridomas after use of the masking, adsorption, and lysis procedures.** When compared to the standard fusion procedure after adjustment to uniform population size (Table 1), the processing of splenocyte cells gave a 27% reduction in total (anti-CaMV and anti-HCC) reactive cells after the use of the masking procedure (treatment 2), reduction of 46% after the adsorption procedure (treatment 4), and of 48% after the lysis procedure (treatment 7). Based on the internal controls, 2 and 11% of these reductions with the masking and lysis procedures, respectively, may be attributed to handling. In the case of the adsorption procedure, the reduction was 11% in the absence of reactive components and 6% when a blocking globulin was used (Table 1).

Inclusion of the masking, adsorption, and lysis procedures each resulted in reduction of total reactive hybridomas (anti-CaMV + anti-HCC) when compared to the standard procedure. Nevertheless, when average results (C.V. = 1-7%) from four

TABLE 1. Production of hybridomas against cauliflower mosaic virus (CaMV) by the standard procedure and by three selective B-cell enrichment procedures<sup>a</sup>

Treatment code no.	Treatment	Fusions (no.)	Total wells with growing hybridomas (no.)	Wells with growing hybridomas (%)	Desired clones (total wells with anti-CaMV clones) (no.)	Undesired clones (total wells with anti-HCC clones) (no.)	Relative % increase (+) or decrease (-) in clones compared to standard procedure <sup>b</sup>	
							anti-CaMV	anti-HCC
1	Standard	4	1729	90	207	718	...	...
2	Immune complex masking	4	1785	93	319	375	+49	-49
3	No immune complex masking	2	883 <sup>c</sup>	92	103	358	-2	-2
4	Immunoadsorption	4	1740	91	359	148	+72	-80
5	Immunoadsorption with rabbit normal globulin	4	1683	85	156	691	-23	-1
6	Noncoated plates	4	1448	75	134	558	-23	-7
7	Complement-mediated cytotoxicity	4	1664	87	307	158	+54	-77
8	Complement with rabbit normal globulin	4	1738	91	133	693	-36	-4
9	No rabbit normal globulin, no complement	4	1636	85	175	659	-11	-3

<sup>a</sup>Treatments are: 1) Standard procedure; 2) B-cells from mouse spleens immunized with a purified preparation of CaMV from Chinese cabbage incubated before injection with an excess of congenic anti-HCC antibodies to give immune complex masking; 3) treatment 2 incubated only with congenic mouse normal globulin; 4) solid-phase immunoadsorption; 5) treatment 4 with normal globulin; 6) noncoated plates; 7) complement-mediated cytotoxicity; 8) complement plus rabbit normal globulin; 9) no rabbit globulin, no complement. HCC = healthy Chinese cabbage antigens.

<sup>b</sup>x of equation 1 in Materials and Methods.

<sup>c</sup>All counts were then doubled to simulate four fusions in order to calculate x.

fusions for each enrichment procedure are adjusted to equal total numbers of hybridomas by equation 1 and compared with the standard procedure (Table 1), it can be seen that all three enrichment procedures caused relative increases in the number of anti-CaMV hybridomas, 49% for masking, 72% for adsorption, and 54% for lysis. Comparable decreases in the numbers of anti-HCC hybridomas were 49, 80, and 77%, respectively. The 72% increase over standard with the adsorption procedure ( $x$  in equation 1 of Materials and Methods) was calculated from Table 1 data as follows:  $x = 100[(359 \times 1,729 / 1,740) - 207] / 207 = 72$ . The adsorption procedure gave the greatest interval (152%) between anti-CaMV hybridoma increase and anti-HCC hybridoma decrease. From Table 1 it can also be seen that each B cell selective enrichment procedure gave more anti-CaMV and fewer anti-HCC hybridomas than its internal controls.

## DISCUSSION

In our investigation, reductions of undesired B-cell populations by immune-complex masking, solid-phase immunoadsorption, or complement-mediated cytotoxicity, and consequent enrichment of desired B-cell populations were evaluated by two methods: PFC assay and hybridoma formation. Because of the variability of the fusion process, its use for quantitative evaluation is limited. Even when precautions are taken to reduce variability through spleen pooling, and a well-defined antigen is employed, the probability of obtaining no antigen-specific hybridomas still exists. However, trends can be discerned if enough fusions are completed and PFC, ELISA, or other such supportive techniques evaluating the splenocyte population as a whole are used.

Using purified CaMV-CC antigens for injection, we observed that  $12 \pm 7\%$  (four tests) of the resulting hybridomas were anti-CaMV, while  $42\% \pm 14\%$  were HCC-specific if the standard fusion procedure was employed. The incorporation of masking, adsorption, and lysis procedures before fusion resulted in percentage occurrence of CaMV hybridoma clones of 18–21% and of HCC hybridoma clones of 9–21% (Fig. 3).

Relative to the standard method, the use of the masking procedure reduced the anti-HCC hybridomas from 42 to 21% and both the adsorption and lysis procedures reduced them from 42 to 9%. Thus, by reducing the undesired anti-HCC B-cell populations, all three enrichment procedures directly reduced the probability that an HCC-programmed splenocyte would successfully fuse with a myeloma or that an HCC-programmed hybridoma would occupy the same well and mask the secretion of a CaMV-

programmed hybridoma clone. These reductions resulted in an increased probability that a successful fusion would occur between a CaMV-specific B cell and a myeloma cell. However, this is not a simple cause-effect relationship since the possibility is also increased for fusions with nonsense-programmed and unprogrammed B cells within the mixture.

Among the three B-cell enrichment procedures, masking, adsorption, and lysis, the method of choice for our laboratory is the adsorption procedure. By incorporating anti-HCC splenocyte immunoadsorption, one step with a total extra processing time of 60 min, we were able nearly to double the number of CaMV hybridomas compared to the standard procedure, with little decrease in viability. As a result, each fusion yielded more desired antigen-specific clones, less time was spent on examining undesirable clones, and there was less masking by undesirable clones. The elimination of B cells by the masking procedure was not as effective as the adsorption procedure, and the lysis procedure reduced the number of desired B cells because of losses due to handling and nonspecific reactions.

The flexibility of the adsorption procedure also allows the investigator to target specific populations of cells, much as is done by fluorescence-activated cell sorting (3). Thus, by use of sufficiently pure antigen, the population of desired B cells could be adsorbed out, fused, and plated to yield an even higher success rate in obtaining desired clones.

When it is difficult to avoid the occurrence of mixed antigens in the development of hybridomas, we feel that the addition of the selective B-cell immunoadsorption procedure to the standard protocol can reduce preparation time, improve fusion efficiency, and increase the number of desirable clones retained after fusion. It is also possible that combinations of the masking, adsorption, and lysis procedures might further reduce the undesired B-cell population.

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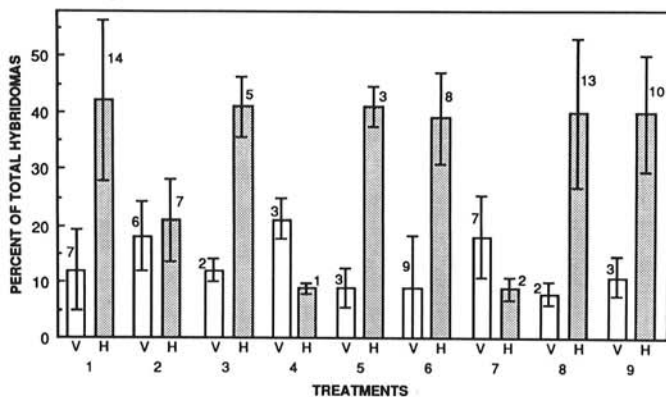


Fig. 3. Percent of total hybridomas per treatment producing antibodies against cauliflower mosaic virus (CaMV, or V) or healthy Chinese cabbage (HCC, or H). Bars are means (each with its standard deviation shown) of four separate experiments. Treatments are: 1) standard procedure; 2) B-cells from mouse spleens immunized with a purified preparation of CaMV from Chinese cabbage incubated before injection with an excess of congenic anti-HCC antibodies to give immune complex masking; 3) treatment 2 incubated only with congenic mouse normal globulin; 4) solid-phase immunoadsorption; 5) treatment 4 with rabbit normal globulin; 6) noncoated plates; 7) complement-mediated cytotoxicity; 8) complement plus rabbit normal globulin; 9) no rabbit globulin, no complement.

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