

STABLE HAMSTER-MOUSE HYBRIDOMAS PRODUCING IgG AND IgM HAMSTER MONOCLONAL ANTIBODIES OF DEFINED SPECIFICITY¹

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Specific antibody-secreting hybridomas have been obtained by fusing Syrian or Armenian hamster (*Mesocricetus auratus* or *Cricetulus migratorius*) spleen cells with mouse myeloma cells. The hamsters were immunized to mouse cytolytic T lymphocytes. Hybrids were selected either by an indirect binding assay using an ¹²⁵I-mono-clonal antibody (MAb) reactive with hamster κ -chains or by their ability to block T cell-mediated cytotoxicity. Three hybridoma clones were obtained that secreted intact IgM-like and IgG-like hamster MAb as shown by SDS-PAGE. The clones were stable as shown by subcloning. Two MAb recognized antigens of wide tissue distribution; the third bound specifically to T lymphocytes, gave strong inhibition of T cell-mediated cytotoxicity, and immunoprecipitated the Lyt-2,3 molecule.

Hybridomas resulting from the fusion of mouse myelomas with mouse or rat spleen cells have been found to be stable sources of mouse and rat monoclonal antibodies (MAb)² of predetermined specificity (reviewed in References 1 and 2). Human, rabbit, and bovine spleen cells have also been used as fusion partners of mouse myeloma cells (3–11). Although secretion of intact antibodies was found in certain cases, a common feature of human \times mouse and rabbit \times mouse hybrid cells was the high rate of chromosome loss and phenotypic instability. Rabbit \times mouse hybrids rapidly lose secretion of rabbit Ig chains, and hybrids secreting both heavy and light chains have only been obtainable with rapid and repeated subcloning (11). Rabbit or bovine MAb of predetermined specificity have not yet been obtained.

In previous studies, we used rat antibodies secreted by rat-mouse hybridomas to study mouse cytolytic T lymphocyte (CTL) cell surface antigens (12, 13). These hybrids were stable and secreted high concentrations of rat MAb. In spite of these advantages, the evolutionary proximity of the rat and mouse may restrict the antibody response. Both species belong to the subfamily Murinae, family Muridae, and thus may share many conserved antigenic structures. To avoid this limitation, hamsters, which are more evolutionarily distant, were immunized with mouse CTL and their spleen cells were fused with mouse myeloma cells to obtain hybridomas secreting hamster MAb.

Although hamster immunology is being increasingly studied (14), hamster MAb have not been obtained, nor have hamster myelomas been available as a source of monoclonal Ig. Hamster hybridomas would be useful in the study of hamster alloantigens, for the definition of hamster Ig subclasses, and for the cloning of hamster Ig genes.

In the present study, species of two different hamster genera (*Cricetidae* family) were found to be excellent fusion partners of mouse myeloma lines and to produce stable hybrids. The successful production of three different hybridoma lines secreting hamster MAb of IgG and IgM classes and the antigen specificities they recognize are described.

MATERIALS AND METHODS

Myeloma lines. The P3X63Ag8 line secretes γ 1 and κ -chains. NS1 and P3X63Ag8.6.5.3 are variant myelomas that synthesize κ only or no chains, respectively (1).

Immunization and fusion. Syrian and Armenian hamsters were obtained from Charles River Laboratories, Wilmington, MA) and Cambridge Diagnostics, Inc. (Cambridge, MA), respectively. They were primed i.p. on day -30 with 10^7 or 5×10^6 C57BL/6J anti-P815 CTL per Syrian or Armenian hamster, respectively. On day -3, the Syrian hamsters were boosted i.v. and the Armenian hamsters i.p. with the identical amount and type of cells. On day 0, NS1 or P3X63Ag8.6.5 mouse myeloma cells were fused with spleen cells from the immunized hamsters using 50% (w/w) polyethylene glycol as described (15, 16). After fusion, hamster peritoneal cells were added as feeder cells to a concentration of 1 to 1.6×10^4 cells/ml in the final hybrid cell suspension in 20% fetal calf serum/Dulbecco's modified Eagle's medium/hypoxanthine/aminopterin/thymidine/2-mercaptoethanol (HAT medium). In fusion M21, spleen cells from one Syrian hamster were fused, one-half with NS1 and one-half with P3X63Ag8.6.5.3 myeloma lines, and were distributed in four 96-well plates. Spleen cells from one Syrian hamster and two Armenian hamsters were fused in parallel with NS1 myeloma cells in fusions M22 (four 96-well plates) and M23 (three 96-well plates) respectively. Cultures were fed by replacing about one-half the medium with HAT medium on days 7 and 11. Beginning on day 14, fusions were fed every 3 days with the same medium lacking aminopterin (HT medium) until they reached near maximal density and began turning the medium yellow, when feeding was carried out every 2 days. After 3 wk, hybridoma culture supernatants were harvested and screened for inhibition of CTL-mediated killing and in the indirect cell-binding assay. Cloning and subcloning in soft agar (1) were carried out for four selected hybrid cell lines. For cloning and subsequent growth, hypoxanthine and thymidine as well as aminopterin were omitted from the above medium. Cloned hybrids could also be grown in RPMI 1640 rather than Dulbecco's modified Eagle's medium and in medium supplemented with horse serum rather than fetal calf serum.

Ig chains. Internal labeling with [¹⁴C]-leucine of products secreted by hamster hybrids, [SD-PAGE] SDS-PAGE of Ig chains, fluorography, and autoradiography were carried out as described previously (17).

CTL and indirect binding assays. Generation of effector cells and inhibition of CTL-mediated cytotoxicity by using C57 anti-P815 (allogeneic) and C57 anti-BNL Φ (xenogeneic) effector cells were performed as reported previously (18). Indirect cell-binding assays to glutaraldehyde-fixed cells or tissue preparations were carried out as described (19). A radiolabeled mouse monoclonal anti-rat κ -chain 1b allotype (RG7/7.6), which is cross-reactive with Syrian and Armenian but not Chinese hamster κ -chains, was used as a second antibody (20). This reagent was purified on *S. aureus* protein A-Sepharose (Pharmacia, Piscataway, NJ) (21) to 80% pure IgG, and then iodinated in solution by the chloroglycoluril method (22).

Immunoprecipitation. Immunoprecipitation was performed in two steps. The hamster MAb were mixed and incubated with labeled cell lysates,

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² Abbreviations used: B6, C57BL/6J; BNL Φ , Brown Norway rat lymphoma; Con A blasts, concanavalin-stimulated spleen cells; CTL, cytolytic T lymphocyte; LFA-1, lymphocyte function-associated antigen 1; LPS blasts, lipopolysaccharide-stimulated spleen cells; MAb, monoclonal antibody(ies); HAT medium, 20% FCS, DMEM, hypoxanthine, aminopterin, thymidine, and 2-mercaptoethanol.

followed by the addition of purified RG7/7.6 MAb coupled to Sepharose (13). Samples were subjected to SDS-PAGE (23) and autoradiography with enhancing screens (24).

Other MAb. In the characterization of the hamster MAb, several rat MAb to mouse cell surface antigens were included for comparison. These included the anti-Lyt-2,3 M12/4 and M12/7 MAb, the anti-Thy-1 M16/1 MAb, and the IgM anti-LFA-1 M18/2 MAb (13).

RESULTS

In preliminary experiments with nonprimed Syrian hamsters and gerbils and with rats as positive controls, hamster and rat but not gerbil hybridomas were obtained and were found to secrete Ig as determined by double immunodiffusion against anti-IgG. Next, spleen cells from Syrian and Armenian hamsters (*Mesocricetus auratus* and *Cricetulus migratorius*) immune to mouse CTL were fused with NSI and P3X63Ag8.6.5.3 mouse myeloma lines. The characteristics of these hamster fusions are shown in Table I. The frequency of hybridomas was twofold greater for Armenian compared to Syrian hamster spleen cells. Growth of 100% of the Armenian hybridoma cultures, which were seeded at 1.4×10^5 spleen cells/culture, showed the fusion frequency was $>0.7 \times 10^{-5}$. This frequency is similar to that for mouse-mouse and rat-mouse fusions (12, 25). In the Syrian but not Armenian hamster fusions, adherent cells with a macrophage-like morphology distinct from B cell hybrids and that did not secrete Ig proliferated in some of the cultures. Depending on the fusion, the percentage of these cultures varied from 20 to 60%. The Syrian and Armenian hamster \times mouse myeloma hybrids grew more slowly during the first weeks than rat \times mouse myeloma hybrids; they reached maximal density at about 3 wk compared to about 2 wk for the rat hybrids. After further growth and cloning, the growth rate appeared similar to that of rat-mouse hybrids. The M23/3 line was found to have a doubling time of 15 hr.

After 3 wk of culture in 96-well plates, the hybrids were screened for binding to Concanavalin A-stimulated spleen cells (Con A blasts) and for their ability to block T cell-mediated cytotoxicity in a xenogeneic killing system (13). The best characterized fusions were M22 and M23. Of 382 growing hybridoma cultures, 26 were positive for binding to Con A blasts and seven for inhibition of CTL-mediated killing in the initial screen. Positive hybrids were transferred to 24-well plates, were grown further, and then retested. On the basis of these assays, seven cultures positive for binding, one of which also blocked killing, were selected for cloning. Active clones were isolated from three cultures. These clones and one clone from the M21 fusion were further characterized.

To examine the Ig chains secreted by the selected hamster hybrids, biosynthetically labeled secreted products were subjected to SDS-PAGE and autoradiography. Three different hamster hybridoma cell lines secreted intact antibody molecules containing hamster heavy and light chains (Fig. 1, lanes 1–6). Two lines, M21/3 and M23/3 (Fig. 1, lanes 1 and 2 and 5 and 6, respectively) produced specific heavy chains of μ -like mobility that migrated similarly to the μ -chain of the rat IgM

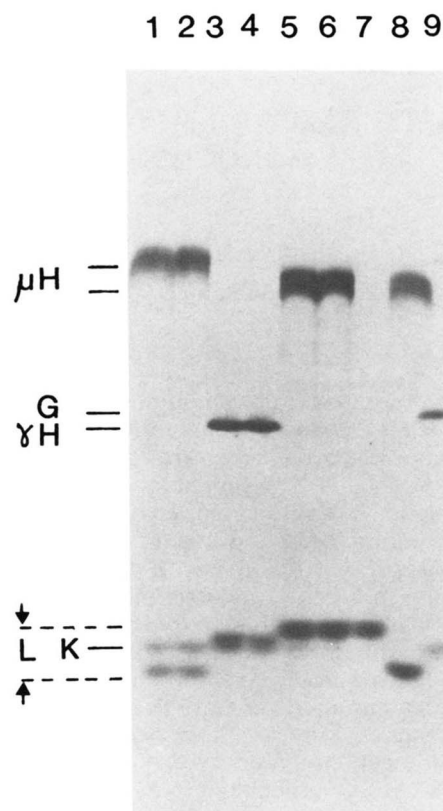


Figure 1. SDS-PAGE characterization of Ig chains secreted by hamster hybrids. Two different clones or subclones of the hamster hybridoma lines M21/3 (M21/3.1.1 and M21/3.2.1, lanes 1 and 2), M22/3 (M22/3.1 and M22/3.2, lanes 3 and 4), M23/3 (M23/3.1 and M23/3.2, lanes 5 and 6), the inactive clone M22/2.1 (lane 7), the IgM κ rat hybridoma line M18/2.9 (13) (lane 8), and the mouse γ 1K myeloma cell line P3X63Ag8 (lane 9) were labeled with [14 C]-leucine (0.1 μ Ci/100 μ l). After labeling, 20 μ l of culture supernatant were subjected to SDS 10% PAGE under reducing conditions and autoradiography. All hybrids were derived from fusions with NSI. μ H and γ H mark the positions of specific heavy chains; L, the specific light chains; G, the myeloma γ -chain; and K the myeloma κ -chain.

M18/2 MAb (Fig. 1, lane 8). Clones of the M22/3 hybrid (Fig. 1, lanes 3 and 4) secreted a heavy chain of γ -like mobility, migrating close to the P3X63Ag8 myeloma γ -chain (Fig. 1, lane 9). These three hamster hybrids secreted specific light chains along with the κ -chain from the mouse myeloma line. Another hybrid, M22/2, which was inactive in the indirect binding assay, secreted only a specific light chain (lane 7).

The cellular distribution of the antigens defined by these MAb was tested with the indirect cell-binding assay. M21/3 and M23/3 MAb recognized antigens of wide cellular distribution, expressed on LPS and Con A-activated spleen cells, thymocytes, nude spleen cells, thioglycollate-induced macrophages, and mouse brain homogenate (Table II). These binding characteristics suggest the antigens defined by these MAb differ from previously described antigens. For example, the T-200 antigen (15) is found on lymphoid cells but not on brain cells, and the Thy-1 antigen (26) (Table II) is found on T lymphocytes but not on B lymphocytes.

In contrast, the M22/3 hamster MAb recognized an antigen expressed selectively by T lymphocytes (Table II). Strong inhibition of CTL-mediated activity was observed in both xenogeneic and allogeneic killing systems in the presence of M22/3 MAb but not the other MAb (Table II). The strong blockade of killing by M22/3 MAb and its cellular reactivity suggested it might recognize the Lyt-2,3 antigen. Immunoprecipitation from 125 I-Con A-stimulated spleen cell lysates followed by SDS-PAGE and autoradiography showed the M22/3 MAb immu-

TABLE I
Hamster \times mouse fusions

Fusion	Hamster Species	Spleen Cells/ Well	Cultures with Hybridomas	Percent
			Total Cultures	
M21	Syrian (<i>Mesocricetus auratus</i>)	2.1×10^5	136/288	47
M22	Syrian (<i>Mesocricetus auratus</i>)	1.6×10^5	190/384	49
M23	Armenian (<i>Cricetulus migratorius</i>)	1.4×10^5	192/192	100

TABLE II
Cell distribution of antigen specificities recognized by hamster MAb and effect on CTL-mediated killing

Hamster MAb	Indirect Binding Assay							Effect on killing ^b			
	Con A Blasts	LPS Blasts	Thymus	Nude Spleen	Brain	mRBC	TG-induced M ϕ	B6 Anti-BNL ϕ		B6 Anti-P815	
								Percent Specific Re-lease	Percent Inhibition	Percent Specific Re-lease	Percent Inhibition
	(radioactivity bound (%)*)										
M21/3	11.6	10.2	9.0	4.1	7.2	0.0	7.1	66	0	52	0
M22/3	3.4	2.0	20.5	0.6	1.1	0.0	0.6	20	69	11	79
M23/3	9.4	7.3	6.8	5.5	5.0	0.3	8.1	73	0	56	0
Rat MAb											
Anti-Lyt-2,3 M12/7	4.1	0.7	11.0	0.4	0.0	0.0	0.9				
Anti-Thy-1 M16/1	9.4	2.2	14.3	0.0	13.3	0.0	0.7				
Background (subtracted)	(.8)	(1.1)	(1.1)	(1.3)	(.9)	(1.6)	(1.7)				

* The percentage of input radiolabeled mouse MAb anti-rat κ -chain RG7/7.6 bound to the different cell types sensitized with hamster MAb was determined in the indirect cell-binding assay. Background binding to cells sensitized with M1/69 HK inactive MAb has been subtracted. Specific binding greater than 2.5% was considered significant.

^b Secondary (1-1) xenogeneic B6 anti-BNL ϕ CTL (E:T = 2:1) and primary (0-1) anti-P815 CTL (E:T = 3:1) were pretreated with an equal volume of hybridoma culture supernatant for 15 min at room temperature, then ⁵¹Cr-labeled target cells were added and the assay was completed. Specific release in the presence of NSI supernatant was 64 and 51%, respectively. The spontaneous release of ⁵¹Cr in wells containing the appropriate culture supernatant but with effector cells omitted was 6.5% and 15%, respectively. Percent of inhibition of specific ⁵¹Cr release is expressed relative to cultures treated with NSI culture supernatant.

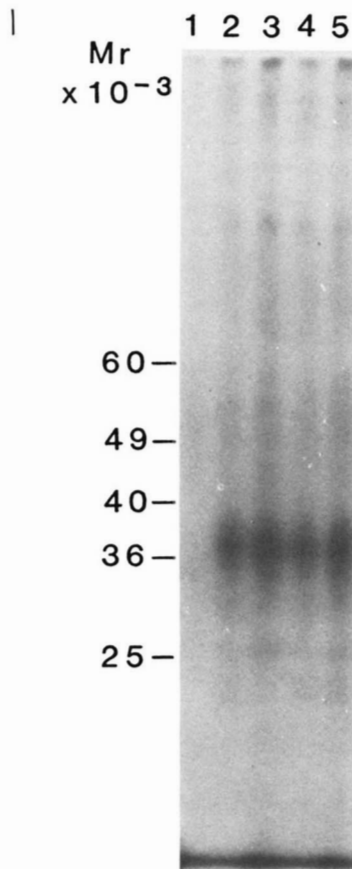


Figure 2. Immunoprecipitation of Lyt-2,3 molecule by hamster MAb. Day 4 Con A blasts were prepared and surface-labeled with ¹²⁵I with Iodogen (22). Cell lysates were immunoprecipitated with 100 μ l of supernatants of NSI plus IgG as control, lane 1; M12/4.2 anti-Lyt-2,3, lane 2; M12/7.2 anti-Lyt-2,3, lane 3; M22/3.1, lane 4; and M22/3.2, lane 5. Immune complexes were precipitated with RG7/7 anti-rat κ -chain MAb coupled to Sepharose. Reduced samples were subjected to SDS 10% PAGE and autoradiography. Mr = m.w.

noprecipitated a molecule of 35,000 m.w. (Fig. 2, lanes 4 and 5). It migrated identically with the Lyt-2,3 molecule precipitated by the anti-Lyt-2,3 M12/4.2 and M12/7 rat MAb (Fig. 2, lanes 2 and 3). Cross-blocking experiments demonstrated that the M22/3 hamster MAb inhibited binding to cells of the M12/4 anti-Lyt-2,3 MAb (not shown), confirming that the M22/3 hamster MAb recognized the Lyt-2,3 molecule.

DISCUSSION

We describe the production of specific antibody-secreting

hybrids between mouse myeloma cells and Syrian or Armenian hamster spleen cells. The hamsters had been immunized to mouse CTL. MAb were selected by the indirect binding assay or by inhibition of CTL-mediated killing. Two MAb were directed to antigens of wide tissue distribution in the mouse, possibly defining previously undescribed antigens. The third MAb blocked CTL-mediated killing and was shown to react with the Lyt-2,3 antigen.

Interspecies hybridomas with mouse myeloma cell lines used as fusion partners with rat, human, rabbit, and bovine spleen cells have been described [2-5, 8-10, 16]. Rat \times mouse hybrids are comparable in stability to mouse \times mouse hybrids. Human \times mouse and rabbit \times mouse hybridomas, however, tend to lose secretion of Ig of the human or rabbit parent rapidly, probably due to loss of the relevant chromosomes. The production and maintenance of hybridomas secreting intact human or rabbit Ig have required rapid and repeated cycles of cloning and selection (6, 7, 11). In contrast, the three hamster hybridoma lines reported in this study were stable after the first cloning. Subclones were 100% active (data not shown) showing these hybridomas do not require multiple cloning cycles to be stabilized. The frequency of fusion for the Armenian hamster was similar to that in rat-mouse cell hybridizations.

These hamster hybridoma cell lines provide the first source of hamster MAb of predefined specificity. Antibodies derived from hamster hybridomas might reveal antigenic determinants that are conserved between mouse and rat, yet are immunogenic in the mouse-hamster combination. Hamster hybridomas should also be useful for obtaining monoclonal alloantibodies. The Syrian hamster has been developed as an immunologic model, and its major histocompatibility complex is being studied (14). The hamster hybridomas reported here are also the first reported source of hamster monoclonal IgG or IgM. They could be used for amino acid sequencing, for the production of serologic reagents, the definition of hamster Ig subclasses, and for cloning of hamster Ig genes.

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