Specific antibody-secreting hybridomas have been obtained by fusing Syrian or Armenian hamster (Mesocricetus auratus or Cricetus 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-monoclonal antibody (MAb) reactive with hamster κ-chains or by their ability to block T cell-mediated cytolysis. Three hybridoma clones were obtained that secreted intact IgM-like and IgG-like hamster MAb as shown by SDS-PAGE. 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

P3X63Ag8.6.5 line secretes only κ-chains. NSI and P3X63Ag8.6.5.3 are variant myelomas that synthesize 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 killed on day -30 with 10% or 5 × 10^5 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, NSI 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 × 10^6 cells/ml in the final hybrid cell suspension in 20% fetal calf serum/Dulbecco's modified Eagle's medium (HAT medium). HAT 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 was carried out for four selected hybrid cell lines.

#### Hybridoma cloning

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) 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 κ mouse and rabbit mouse hybrid cell lines was the high rate of chromosome loss and phenotypic instability. Rabbit κ 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-human 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, 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 (Mesocricetus auratus 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.** P3X63Ag8.6.5 line secretes κ-chains only or no chains. NSI and P3X63Ag8.6.5.3 are variant myelomas that synthesize only κ-chains 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 killed on day -30 with 10% or 5 × 10^5 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, NSI 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 × 10^6 cells/ml in the final hybrid cell suspension in 20% fetal calf serum/Dulbecco's modified Eagle's medium (HAT medium). In fusion M21, spleen cells from one Syrian hamster were fused, one-half with NSI 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 NSI 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.
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 IgG as determined by double immunodiffusion against anti-lgG. 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 x 10^5 spleen cells/culture, showed the fusion frequency was >0.7 x 10^-3. 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 IgG 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 x mouse myeloma hybrids grew more slowly during the first weeks than rat x 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 cytolysis 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 these cultures. These clones and one clone from the M21 fusion were further characterized.

To examine the Ig chains secreted by the selected hamster hybridomas, 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 5 and 6, respectively) produced specific heavy chains of μ-like mobility that migrated similarly to the μ-chain of the rat IgM

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>Hamster x mouse fusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusion</td>
<td>Hamster Species</td>
</tr>
<tr>
<td>----------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>M21</td>
<td>Syrian (Mesocricetus auratus)</td>
</tr>
<tr>
<td>M22</td>
<td>Syrian (Mesocricetus auratus)</td>
</tr>
<tr>
<td>M23</td>
<td>Armenian (Cricetulus migratorius)</td>
</tr>
</tbody>
</table>

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), M21/3 (M21/3.1 and M21/3.2, lanes 5 and 6), the inactive clone M22/2.1 (lane 7), the IgMc rat hybridoma line M18/2.9 (13) (lane 8), and the mouse γ1K myeloma cell line P3X63Ag8 (lane 9) were labeled with [35S]-leucine (0.1 μCi/100 μl). After labeling, 20 μl of culture supernatant were subjected to SDS 10% PAGE under reducing conditions and autoradiography. All hybrids were derived from fusions with NSI, μH and γH mark the positions of specific heavy chains; L, the specific light chains; G, the myeloma γ-chain; and K the myeloma κ-chain.

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 125I-Con A-stimulated spleen cell lysates followed by SDS-PAGE and autoradiography showed the M22/3 MAb immu-
HAMSTER MONOCLONAL ANTIBODIES TO MOUSE CTL ANTIGENS

### TABLE II

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

<table>
<thead>
<tr>
<th>Hamster MAb:</th>
<th>Indirect Binding Assay</th>
<th>Effect on killing$^2$</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Con A Blasts</td>
<td>LPS Blasts</td>
<td>Thymus</td>
<td>Nude Spleen</td>
<td>Brain</td>
<td>mRBC</td>
</tr>
<tr>
<td>M21/3</td>
<td>11.6</td>
<td>10.2</td>
<td>9.0</td>
<td>4.1</td>
<td>7.2</td>
<td>0.0</td>
</tr>
<tr>
<td>M22/3</td>
<td>3.4</td>
<td>2.0</td>
<td>20.5</td>
<td>0.6</td>
<td>1.1</td>
<td>0.0</td>
</tr>
<tr>
<td>M23/3</td>
<td>9.4</td>
<td>7.3</td>
<td>6.8</td>
<td>5.5</td>
<td>5.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Rat MAb</td>
<td>Anti-Lyt-2,3 M12/7</td>
<td>4.1</td>
<td>0.7</td>
<td>11.0</td>
<td>0.4</td>
<td>0.0</td>
</tr>
<tr>
<td>Anti-Thy-1 M16/1</td>
<td>9.4</td>
<td>2.2</td>
<td>14.3</td>
<td>0.0</td>
<td>13.3</td>
<td>0.0</td>
</tr>
<tr>
<td>Background (subtracted)</td>
<td>(8)</td>
<td>(1.1)</td>
<td>(1.1)</td>
<td>(1.3)</td>
<td>(0.9)</td>
<td>(1.6)</td>
</tr>
</tbody>
</table>

* The percentage of input radiolabeled mouse MAb anti-rat x-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.

* Secondary (1-1) xenogeneic B6 anti-BNLg 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 loldogen (22). Cell lysates were immunoprecipitated with 100 μl 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 x-chain MAb coupled to Sepharose. Reduced samples were subjected to SDS 10% PAGE and autoradiography, Mr = m.w.

nproprecipitated 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 hybrids 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 x mouse hybrids are comparable in stability to mouse x mouse hybrids. Human x mouse and rabbit x mouse hybridomas, however, tend to lose secretion of lg 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 lg 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 allogeneic antibodies. 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 lg subclasses, and for cloning of hamster lg genes.

### REFERENCES

HAMSTER MONOCLONAL ANTIBODIES TO MOUSE CTL ANTIGENS


