# [20] Production of Syrian and Armenian Hamster Monoclonal Antibodies of Defined Specificity

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Interspecies hybridomas have been generated using mouse myeloma cell lines as fusion partners with rat, human, rabbit, and bovine spleen cells. Rabbit × mouse hybrids rapidly lose secretion of rabbit Ig chains, probably due to the high rate of chromosome loss.[1] In contrast, fusions of mouse myeloma cells with mouse or rat spleen cells yield mouse and rat hybridomas which represent stable sources of mouse and rat monoclonal antibodies (MAbs).[2,3] Rat MAbs secreted by rat–mouse hybrids have been utilized to study mouse cell surface antigens.[3,4] However, identification of some surface molecules could be hampered by the evolutionary proximity of the rat and mouse. Many antigenic structures may remain conserved in these two species, which belong to the same Murinae subfamily.

In the search for animals evolutionarily distant from the mouse, we found that B lymphocytes from two different species of hamster (Cricetidae family) are excellent fusion partners with mouse myelomas.[5] Here, the production of hamster MAbs is described. Since the methods are in great part identical to those for mouse and rat MAbs, only the variations are described. A MAb reactive with Armenian and Syrian κ chains, which is a very useful screening reagent, is additionally described.

## Generation and Characterization of Hamster–Mouse Hybridomas

### *Parental Cells*

*Myeloma Lines.* The characteristics of mouse myeloma lines used in fusion experiments have been described in detail.[2] NSI (NSI/1.Ag4.1) and P3X63Ag8.653 are variant myelomas that synthesize κ light chain or no

[1] J. A. Sogn, M. C. Kuo, and T. J. Kindt, *Fed. Proc.* **41**, 595 (1982).
[2] G. Galfré and C. Milstein, this series, Vol. 73, p. 3.
[3] R. H. Kennett, T. J. McKearn, and K. B. Bechtol, "Monoclonal Antibodies." Plenum, New York, 1980.
[4] T. A. Springer, D. Davignon, M. K. Ho, K. Kürzinger, E. Martz, and F. Sanchez-Madrid, *Immunol. Rev.* **68**, 111 (1982).
[5] F. Sanchez-Madrid, P. Szklut, and T. A. Springer, *J. Immunol.* **130**, 309 (1983).

chain, respectively. These parental lines are maintained in logarithmic growth in suspension cultures or in spinner cultures. As medium for growth, we use either RPMI 1640 or Dulbecco's modified Eagle's medium (DME) supplemented with glutamine and 10% fetal calf serum (FCS).

*Immunization of Animals.* Syrian (*Mesocricetus auratus*) and Armenian (*Cricetulus migratorius*) hamsters can be obtained from Charles River Laboratories (Wilmington, MA) and Cambridge Diagnostics (Cambridge, MA 02139) or Dr. George Yerganian, Newton-Wellesley Hospital (Newton, MA 02162), respectively. Outbred animals from 2 months to 1 year old are used. Animals usually are primed with antigen intraperitoneally on day −30 and boosted intravenously on day −3 prior to fusion on day 0. For soluble antigens which equilibrate between extravascular and vascular spaces, it may be more convenient to boost intraperitoneally. Relative to mice, Armenian hamsters have short (2-in.), puny tails. Unless caged individually after the age of 8 weeks, female Armenian hamsters bite one another's tails off. Males are less aggressive. If tails are present, investigators with skill in the technique can successfully inject in the tail vein. Alternatively, boosting in the jugular vein is described.

*Materials*

Xylazine (Rompun; Haver-Lockhart, Cutter Labs, Shawnnee, KA 66201)
Ketamine (Ketaset; Bristol Labs, Syracuse, NY 13201)
Anesthetic: 1.6 mg Xylazine/ml, 1.6 mg ketamine/ml, in sterile water, in 1-ml syringe
27-gauge needles
1-ml syringe containing antigen in 0.2 ml saline
Tweezers and scissors
Wound clips (autoclip, Clay-Adams)

*Procedure.* Inject 0.1-ml aliquots of anesthetic intramuscularly with a 27-gauge needle. Wait several minutes after each injection, and test for lack of any response to squeezing the foot with tweezers. Typically, this occurs after about 0.3 ml for Armenian hamsters (about 30 g). Make a vertical incision along sternocleido-mastoid line using blunt-pointed scissors, exposing the submaxillary and sublingual glands. These are separated with blunt scissors to expose the jugular vein. Injection is with a 27-gauge ½-in. needle, bent slightly to facilitate a more shallow penetration of the vein. The incision is then cleaned and closed with 9-mm wound clips.

*Fusion*

The fusion procedure was not varied from the standard conditions for mouse–mouse and rat–mouse fusions.[2,6,7] Briefly, 50% (w/w) PEG (polyethylene glycol, MW 1,500, BDH) was prepared by autoclaving 10 g, cooling to 50°, and adding 10 ml of DME. The pH was adjusted to pH 7.4. The solution was prepared at least 1 day before fusion and could be kept at least 2 months. On day 0, hamsters were killed with $CO_2$ in a chamber with dry ice and water, and washed with 70% ethanol. Peritoneal cells were removed by intraperitoneal lavage with 10 ml or 5 ml of 10 units heparin/ml in PBS per Syrian or Armenian hamster, respectively. Hamster spleens were removed and cell suspensions prepared and fused with NSI or P3X63Ag8.653 mouse myeloma cells at a 1 : 4 myeloma to spleen cell ratio according to the Galfré procedure.[2,6,7] Syrian hamsters yielded about $6 \times 10^7$ white cells/spleen. Armenian hamsters yielded $2 \times 10^7$ to $10^8$ white cells/spleen. After fusion, cells were resuspended directly in HAT selection medium and distributed in flat-bottomed microculture 96-well plates (Costar, No. 3596) at $2 \times 10^5$ spleen cells per well. Hamster peritoneal cells were added as feeder cells at a concentration of $2 \times 10^4$ cells/ml in the final hybrid cell suspension.

*Growth Characteristics of Hybrids*

The feeding schedule of cultures is similar to that described for rat–mouse hybrids.[5] Cultures are fed by replacing about one-half the medium on days 7 and 11 after fusion. After 2 weeks, cultures are fed every 3 days with the same medium lacking aminopterin (HT medium) until the medium becomes yellow. Then, feeding is carried out every 2 days. Visible growth is observed 1 week after fusion.

Compared to mouse and rat hybrids, Armenian hamster hybrids grow at a similar or slightly slower rate, and Syrian hamster hybrids grow somewhat more slowly during the first 2 weeks. After cloning (in soft agar), growth rates of both types of hamster hybrids are excellent. The doubling time was measured for one Armenian hybrid and found to be 15 hr. The frequency of wells with growing hybridomas was measured in four independent fusions[5] (and unpublished). Hybridomas grew in 50% of Syrian hybrid cultures seeded with 1.6 to $2.1 \times 10^5$ spleen cells and in

[6] G. Galfré, S. C. Howe, C. Milstein, G. W. Butcher, and C. J. Howard, *Nature (London)* 266, 550 (1977).

[7] Z. Eshhar, *in* "Hybridoma Technology in the Biosciences and Medicine" (T. A. Springer, ed.), p. 3. Plenum, New York, 1985.

100% of American hybrid cultures seeded with 1.4 to $5 \times 10^5$ cells. Thus, the frequency of Armenian hybrids is $>0.7 \times 10^{-5}$, which is similar to that of murine hybrids.

In Syrian hamster fusions, adherent cells with a fibroblast-like morphology proliferated in 20–60% of the cultures. These cells were readily distinguished morphologically from the round, nonadherent B-cell hybrids and did not secrete Ig. Proliferation was so vigorous that this cell type appeared to often overwhelm the hybridomas. Such rapidly proliferating adherent cells were not observed in Armenian fusions in our hands, but have been found by others, and overgrew the hybridomas (J. Unkeless, U. Rockefeller, and R. Schreiber, Scripps Clinic, La Jolla, CA 92037). Thus far, the reasons for the predominance of this undesirable cell type in some but not other fusions are not clear. It is possible that the fusion conditions or growth factors in serum influence its appearance. Growth of this fibroblast-like cell type has not been seen in fusions with mouse or rat spleen cells.

### *Cloning*

Hamster hybrids secreting antibodies of desired specificity are cloned in soft agar or in microtiter wells.[2] Stability has been measured by recloning and determining the percentage of active subclones. Subclones are almost always 100% active, showing hamster hybridomas are at least as stable as mouse or rat hybridomas.

### *Screening*

The usual types of screening procedures can be applied to hamster MAbs. We have selected for MAbs which inhibit T-lymphocyte-mediated killing in functional screening, and have also used an indirect cell binding assay.[5] A potential disadvantage of the hamster is the limited commercial availability of antibodies to hamster Ig. Antisera to Syrian hamster (usually listed simply as "hamster" or "golden hamster") but not Armenian hamster IgG are available.

Fortunately, an anti-hamster Ig MAb has been produced which serves as an excellent reagent for use with hamster MAbs.[8] Labeled with $^{125}I$, it can be used as a second antibody[8] to detect hamster MAbs of desired antigen specificity. It can be coupled to Sepharose to purify Ig or to isolate antigen–hamster MAb complexes for biochemical analysis of antigens.[5] This MAb, RG7/7, was originally selected as an antibody to rat $\kappa$ lb

[8] T. A. Springer, A. Bhattacharya, J. T. Cardoza, and F. Sanchez-Madrid, *Hybridoma* **1**, 257 (1982).

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| MAb | Class[a] | Hamster | Antigen polypeptide chain(s)[b] (MW × $10^{-3}$) | Antigen name | Cell distribution[c] |
|---|---|---|---|---|---|
| M21/3 | IgM | Syrian | — | — | B and T lymphocytes, macrophages, brain |
| M22/3 | IgG | Syrian | 35 | Lyt-2,3 | T lymphocytes |
| M23/3 | IgM | Armenian | — | — | B and T lymphocytes, macrophages, brain |
| M24/1 | ND[d] | Armenian | 180, 95 | LFA-1 | Leukocytes |
| M24/2 | ND | Armenian | 25 | Thy-1 | T lymphocytes, brain |
| M24/5 | ND | Armenian | 200, 160 | — | T and B lymphocytes, macrophages |
| M24/6 | ND | Armenian | 12 | — | Lymphocytes |
| M24/8 | ND | Armenian | 25 | Thy-1 | T lymphocytes, brain |

[a] Determined by mobility of heavy chains in SDS–PAGE.
[b] Determined by SDS–PAGE of reduced, $^{125}$I-labeled, immunoprecipitated antigen.
[c] Determined by indirect binding assay with $^{125}$I-RG7/7.[8]
[d] Not done.

isotype light chains, but binds with even higher affinity to κ light chains of Armenian and Syrian hamster Ig.[5,8] It does not cross-react with Chinese hamster, human, rabbit, guinea pig, or mouse IgG. Four of four hamster MAbs which we identified by functional screening reacted in indirect binding assays with this MAb. It thus appears that most hamster MAbs contain the κ light chain, in similarity to the mouse and rat, where the κ to λ ratio is >9:1. The RG7/7 anti-Syrian and Armenian hamster κ chain hybridoma cell line may be obtained from the American Type Culture Collection, 12301 Parklawn Dr., Rockville, MD 20852. Purified RG7/7 IgG may be obtained from Boehringer-Mannheim or Hybritech, 11085 Torreyona Rd., San Diego, CA 92121. RG7/7 is a mouse $IgG_{2a}$ MAb, and can be purified on *Staphylococcus aureus* protein A–Sepharose.[9]

Hamster Ig subclasses are only in the initial stages of characterization. Syrian hamster $IgG_1$ and $IgG_2$ have been reported to both bind to protein A at pH 8 and to be differentially eluted at low pH.[10] Syrian hamster Ig binds to protein A with an affinity intermediate between that of rat and mouse Ig.[11] IgM and IgG hamster hybridomas can be differentiated by the

[9] P. L. Ey, S. J. Prowse, and C. R. Jenkin, *Immunochemistry* **15**, 429 (1978).
[10] M. J. Escribano, H. Haddada, and C. De Vaux Saint Cyr, *J. Immunol. Methods* **52**, 63 (1982).
[11] D. D. Richman, P. H. Cleveland, M. N. Oxman, and K. M. Johnson, *J. Immunol.* **128**, 2300 (1982).

mobility of their heavy chains in SDS–PAGE.[5] Secreted Igs can be conveniently analyzed after labeling with radioactive amino acids.

## Conclusion

A number of hamster MAbs have thus far been obtained which recognized mouse T-lymphocyte surface antigens (table). Hamster MAbs to mouse $\gamma$-interferon have also been obtained.[12] Hamster hybridomas are an excellent and stable source of MAbs of predefined specificity. Their primary use thus far has been for the preparation of anti-mouse MAb, but they have the potential for much wider applications.

## Acknowledgments

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[12] R. D. Schreiber, L. J. Hicks, A. B. Celada, and P. W. Gray, *J. Immunol.* **134**, 1609 (1985).

# [21] Production and Characterization of Bovine Immunoglobulins from Bovine × Murine Hybridomas

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## Introduction

Characterization of the classes and subclasses of immunoglobulins (Ig) is important for the study of humoral immune response and the structure–function relationship between Ig classes and subclasses and effector mechanisms. In human, murine, and a few other species, such characterization has been facilitated by the availability of homogeneous Ig produced by multiple myelomas.[1] Except for an isolated report of the appearance of Bence-Jones proteins in the bovine[2] species there have been no reports of bovine myelomas to date. The lack of availability of myelomas has made the characterization of bovine Ig difficult. Characterization of bovine Ig has been based on studies made with heterogeneous Ig preparations purified from serum, colostrum, and other exocrine secretions, em-

[1] M. Potter, *Adv. Immunol.* **25**, 141 (1977).
[2] S. Rodkey and A. T. Kimmel, *Immunochemistry* **9**, 23 (1972).