Fig. 2. Dosage-dependent reduction by dexamethasone of labeling of endothelial cells by tritiated thymidine in rat retinal venules following lens puncture. Each value represents mean and S.E. from four experimental animals (eight eyes) that received dexamethasone intraperitoneally twice daily at the indicated dosage.

and the mechanism by which dexamethasone inhibits endothelial cell proliferation following ocular trauma remain to be determined.

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Key words: neovascularization of retina, retinal vessels, dexamethasone

REFERENCES

Synthesis of gamma crystallin by a cloned cell line from Nakano mouse lens. P. Russell, D. A. Carper, and J. H. Kinoshita

A cloned cell line was derived from a culture of Nakano mouse lens epithelial cells. The cloned cells grew vigorously and produced large numbers of lentoid bodies. Sodium dodecyl sulfate (SDS) and non-SDS slab-gel electrophoresis of the soluble proteins from the cultured cells revealed protein bands identical in pattern to those of purified gamma crystallin. Antibody to mouse gamma crystallin reacted to the soluble protein fraction of the cultured cells, indicating the synthesis in culture of gamma crystallin by this cloned cell line.

One method of obtaining homogeneity of tissue culture cells is the technique of cloning. By isolating and subsequently culturing single cells, cell lines with genetic uniformity are established. These cloned cell lines can be further studied for various properties. We have previously reported on the culture of lens epithelium from normal and Nakano mice. The Nakano mouse develops a hereditary cataract and offers an excellent model for the study of human congenital cataracts. In this study, a clone from a cell line of Nakano lens epithelium was selected and checked for the presence of gamma crystallin. The synthesis of this protein has been reported to occur in the lens fibers and not in the epithelium. Thus the presence of gamma crystallin in cultured cells is a possible indication that differentiation of the lens cells has taken place. The techniques of slab-gel electrophoresis and double immunodiffusion were utilized to determine the presence of this crystallin in the cloned cell line.
Methods. The epithelial cells from Nakano mouse lens culture NKR-11 were maintained as previously described. Clones from NKR-11 were obtained by the microtest plate method. Clone NKR-11J was selected for this study because of the vigorous growth of the cells as well as the large number of lentoid bodies present in the culture. The cells were subcultured with a split ratio of 3:1.

For biochemical studies, the cells were washed twice with buffered saline, harvested by scraping, and homogenized in water. The homogenate was centrifuged at 48,000 x g for 30 min. and the soluble fraction was used for electrophoretic and immunodiffusion studies. Protein concentrations were determined for each sample. Discontinuous polyacrylamide slab gels, run at constant current, were prepared according to the method of Maizel. For gels with and without sodium dodecyl sulfate (SDS), the concentrations of acrylamide were 5% in the spacer gel and 13% in the resolving gel. The electrode buffer used was 0.05M Tris-glycine, pH 8.9, with 1% 2-mercaptoethanol. For SDS electrophoresis, the electrode buffer, in addition, contained 0.1% SDS. Each sample well received approximately 10 μl of sample ranging in concentration from 100 to 250 μg per well. For SDS gels, the samples were heated with 1% SDS for 90 sec at 100°C. Bovine serum albumin, ovalbumin, chymotrypsin, and insulin were utilized as standards. Gels were stained with Coomassie brilliant blue. Alpha, beta, and gamma crystallin were purified by Sephadex G-200 column chromatography. Each protein was rechromatographed twice.

Antibody to mouse gamma crystallin was obtained from sheep. Double immunodiffusion was used to test the reactivity of NKR-11J soluble fraction and of purified alpha, beta, and gamma mouse crystallins to antibody to mouse gamma crystallin. The preparation of this antibody has been reported.

Results. Our previous study on cultured lens epithelium demonstrated by fluorescent antibody techniques that gamma crystallin was associated with cells in the vicinity of the lentoid body. Only the cells near the lentoid body had measurable fluorescence. Other cells not in the vicinity of these structures did not show a positive reaction with this technique. Cloning of cell line NKR-11 was attempted, since the presence of gamma crystallin in these cultures had been observed by immunofluorescence. Numerous cloned cell lines were developed, and one, NKR-11J, was selected because of the numerous lentoid structures present in vitro. The cloned cells grew vigorously, yielding approximately 1 mg of soluble protein per 75 cm² tissue culture flask.
Fig. 3. Double immunodiffusion with antibody to mouse gamma crystallin. 1, Mouse alpha crystallin; 2, mouse beta crystallin; 3, mouse gamma crystallin; 4, soluble proteins of cell line NKR-11J; 5, soluble mouse lens proteins.

On non-SDS slab gels, the NKR-11J soluble fraction and the whole mouse lens soluble fraction revealed a complex protein pattern (Fig. 1). There were several bands at similar electrophoretic mobility. Two of these bands appeared to correspond to the two bands of purified mouse gamma crystallin. On SDS slab gels, NKR-11J, mouse lens, and gamma crystallin had a predominant band in common (Fig. 2); moreover, this band was approximately 18,000 MW as determined by protein markers. Thus, for both non SDS and SDS gels, a protein of similar electrophoretic mobility and molecular weight was present in the samples of cell line NKR-11J, whole mouse lens, and purified gamma crystallin.

However, to prove more conclusively that gamma crystallin was being produced by NKR-11J, immunodiffusion tests were run. Antibody to mouse gamma crystallin reacted with whole mouse lens homogenate and purified mouse gamma crystallin. No reaction was observed with purified alpha or beta crystallin (Fig. 3). The strong reaction of the antibody to the soluble fraction of NKR-11J confirmed that this crystallin was produced in the cloned cell cultures.

Discussion. Cloning of lens epithelium in tissue culture assures a homogeneity of genetic information for a cell line. This uniformity of the cloned cells is useful in the study of the proteins of the lens only if the cloned cells retain certain characteristics of lens cells. One of the important characteristics that is an indication of differentiation of lens cells is the ability to synthesize gamma crystallin. In previous studies, we have observed that gamma crystallin was associated with the lentoid bodies of lens epithelial cells in culture and that some morphological differentiation occurred in the lentoid body. Cloning of lens epithelium resulted in cell lines also synthesizing gamma crystallin. The banding pattern of the cloned cells showed that both with and without SDS, the gamma crystallin synthesized was similar in electrophoretic properties and molecular weight to that found in normal mouse lens. The immuno diffusion data confirmed that these bands from NKR-11J were indeed gamma crystallin.

Since some characteristics of lens cells are maintained in cloned cells, further investigation of biochemical events in maturation and cataract development can be undertaken. The technique of polyacrylamide slab-gel electrophoresis offers a useful method to study proteins from lens epithelial cells from not only the mouse but also human lens cells in tissue culture.


Key words: Nakano mouse, cloned cells, gamma crystallin, slab gel electrophoresis, immunodiffusion

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