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NOTES

Aleutian Disease of Mink: Production of ^{14}C-Labeled Antiviral Antibodies by Mink Lymphoid Cells In Vitro

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Lymphoid cells from mink infected with Aleutian disease virus produced labeled, specific antiviral antibody when incubated in medium containing [^{14}C]tyrosine.

Aleutian disease (AD) of mink is a viral-induced, immune complex disease characterized by persistent viremia and the production of large amounts of nonneutralizing antiviral antibody (1, 2, 4, 5, 10; M. E. Bloom, R. E. Race, W. J. Hadlow, and B. Chesebro, J. Immunol., in press). One feature of AD is that numerous aberrations of both humoral and cellular immunity occur (6, 7, 9). In vitro analysis of the primary immune response to Aleutian disease virus (ADV) could be a useful tool in elucidating the mechanisms involved. In several systems, the Mishell-Dutton culture technique has provided an insight into basic immune mechanisms (8), but the general applicability of this method has been limited by the lack of a suitable assay for in vitro primary immune responses. This note describes a technique that may provide a satisfactory assay in a Mishell-Dutton system for ADV. By cultivating lymphoid cells from infected mink in [^{14}C]tyrosine-containing media and assaying the supernatants by a combination of counterimmunoelectrophoresis (CIEP) and autoradiography, it can be shown that these cells produce labeled, specific antiviral antibody.

Eight female mink of the pastel color phase (4) were studied; four were inoculated intraperitoneally with 100 mean lethal doses of Utah-I ADV in a splenic suspension, two were inoculated with the control suspension, and two served as unmanipulated controls. Only the four mink inoculated with ADV had pathological and serological evidence of AD (4; Bloom et al., in press).

Single-cell suspensions containing 5×10^6 viable lymphoid cells were prepared from lymph nodes and spleens in tyrosine-deficient RPMI 1640 containing 5% fetal calf serum, antibiotics, and glutamine (all from GIBCO, Grand Island, N.Y.). Portions (1 ml) of the cells were placed in 35-mm petri dishes with 2 μCi of [^{14}C]tyrosine (L-[U-^{14}C]tyrosine, 300 mCi/mmol; ICN, Irvine, Calif.), and the cultures were incubated overnight at 37 C. Several cultures were incubated for 4 days prior to addition of [^{14}C]tyrosine.

After the incubation period, the contents of the petri dishes were freeze-thawed and centrifuged to remove debris. Supernatants were dialyzed against saline and concentrated with Minicon-S serum concentrators (Amicon Corp., Lexington, Mass.).

CIEP was performed as described previously (Bloom et al., in press). Anodal wells were charged once with a mink carrier serum known to have an antibody titer in CIEP of 1:1,024 to ADV; the anodal wells were then charged twice with concentrated culture supernatants. Cathodal wells were filled with the viral or control antigen, and CIEP was run for 45 min at 6 V/cm. Slides were washed, dried, and stained with azocarmine G.

For immunoelectrophoresis (IEP) of mink serum proteins, standard methods were used (3). Wells were charged once with carrier serum and twice with culture supernatants. Electrophoresis was for 1 h at 6 V/cm, and precipitin lines were developed for 48 h with an anti-whole mink serum prepared in rabbits (gift of John Coe). The slides were then washed, dried, and stained.

For production of autoradiographs (3), the slides were apposed to sheets of Royal Pan film (Eastman Kodak, Rochester, N.Y.) and

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exposed for 2 weeks. The film was developed in Kodak D-11, fixed in Kodak Rapid-Fix, washed, and dried. Photographic enlargements were made of corresponding slides and sheets of film for comparison.

When autoradiography was performed on CIEP preparations, the supernatants from cultures prepared from infected mink produced autoradiographs of precipitin lines (Fig. 1). Culture supernatants from both spleens and lymph nodes produced definite autoradiographs. The images were sharp and well localized to the precipitin line. Autoradiographs were also obtained when cells from infected animals were incubated 4 days prior to the addition of [^{14}C]tyrosine. None of these supernatants produced autoradiographs when tested against the control CIEP antigen.

When culture supernatants prepared from the control mink were assayed against the viral or control antigen, no autoradiographs were produced (Fig. 1).

To exclude nonspecific adsorption of labeled proteins to precipitin lines, culture supernatants previously shown to be positive were incorporated into a CIEP assay not involving ADV, in which ultraviolet light-inactivated herpes simplex virus type I (HSVI) and rabbit anti-HSVI (gift of Donald Lodmell) were used as the antigen and antibody, respectively. No autoradiographic labeling of the HSV—anti-HSV precipitin lines was observed.

All culture supernatants, when tested in IEP and processed for autoradiography, produced autoradiographs of precipitin lines corresponding to 7S immunoglobulin G (3) (Fig. 2). Such

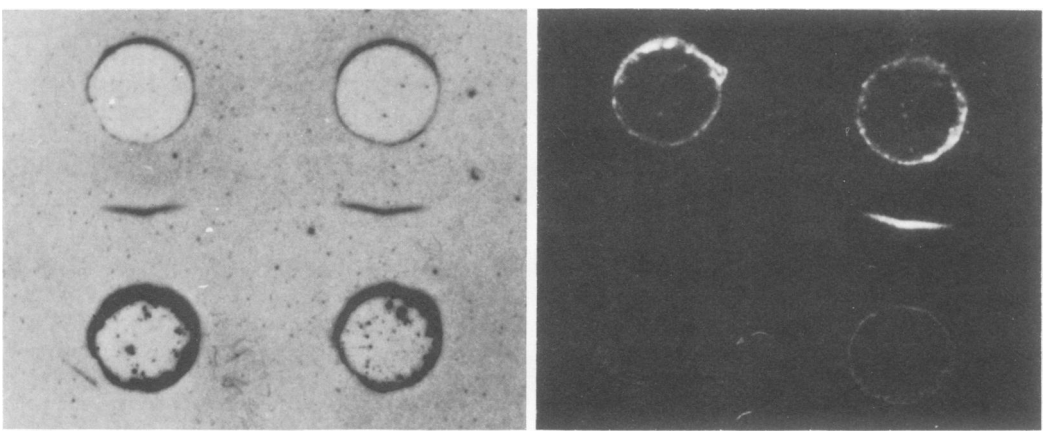

FIG. 1. *CIEP and respective autoradiograph of cell culture supernatants. (Left) CIEP preparation stained with azocarmine G. Both anodal (upper) wells were charged with carrier serum containing antibody against ADV and then charged with concentrated culture supernatants. The well on the left was charged with spleen cell culture supernatant of normal mink; the well on the right was charged with culture supernatant from mink inoculated with ADV. Cathodal (lower) wells were charged with viral antigen and reacted as described in the text. Since an antibody-containing carrier serum was used in both anodal wells, precipitin lines were formed in each case. (Right) Autoradiograph of the same CIEP preparation. Note that only the culture supernatant from the mink infected with ADV produced an autoradiograph.*

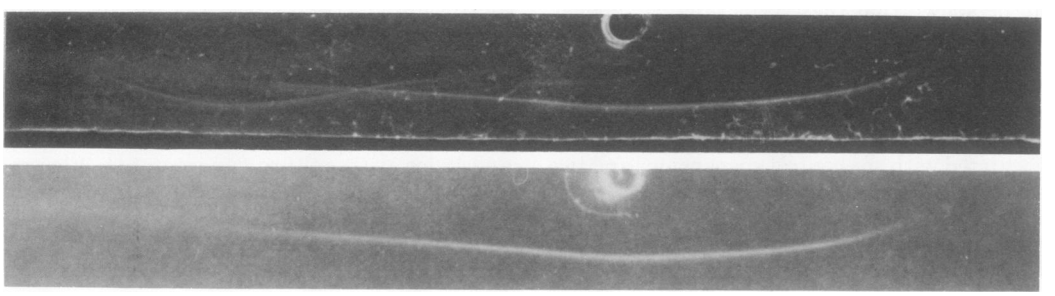

FIG. 2. *IEP pattern (upper) and respective autoradiograph (lower) of supernatant from spleen cell culture prepared from mink inoculated with ADV. The well was charged with a carrier serum and then with culture supernatant prior to processing as described in the text. The autoradiograph shows labeling of proteins corresponding to 7S immunoglobulin G.*

evidence of immunoglobulin synthesis was obtained from both infected and control animals and both lymph nodes and spleens.

These experiments have shown that lymphoid cells from mink infected with ADV produced radioactive immunoglobulin when cultured in the presence of [^{14}C]tyrosine. With a combination of autoradiography and CIEP, some of this immunoglobulin was shown to have antibody activity against ADV. Cells from normal mink also produced immunoglobulin, but no antibody activity could be detected.

These results show that viable lymphoid cells of mink retain the ability to synthesize antibody in vitro. Since cells cultured for 4 days were able to produce antibody when incubated with [^{14}C]tyrosine, production of labeled antibody may be a suitable assay for a primary in vitro immune response against ADV. Such studies are currently under way in this laboratory.

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