Immunofluorescence and Virus Recovery: Correlation in a Murine Leukemia System

Henry C. Orr, John B. Moloney, Joel I. Reisher, and Harry F. Bushar
Bionetics Research Laboratories, Inc., Kensington, Maryland

Summary

Results from mouse infectivity assays and immunofluorescent examinations during pathogenesis of the Moloney murine leukemia virus infection in adult BALB/c mice indicated that the two detection methods could be only partially correlated. The fluorescent antibody technic detected viral antigens as early as 4-5 days after virus inoculation, although infectious virus could not be extracted from the same tissues until the 16th day of the infection. In terms of infectivity titers, immunofluorescence detected less than one-half the log of a 50% infectious dose of virus. Cells from either spleen or peripheral blood of inoculated mice reacted identically in the fluorescent antibody tests.

Introduction

Use of the fluorescent antibody technic in searching for candidate viruses of etiologic significance in human leukemia and lymphomas followed successful realization of its potential in murine viral leukemia systems (4, 8). However, there is little information, especially from in vivo studies, relating immunofluorescence and infectivity titers of the virus. This knowledge would aid in determining the sensitivity of the technic when applied to viral leukemia systems. The present study attempted to correlate immunofluorescence and recoverable virus from mice during pathogenesis of the Moloney leukemia virus infection.

Materials and Methods

Mouse Infectivity Assay. Weanling BALB/c mice of both sexes were each injected, intraperitoneally with 0.25 ml of a 10^{-2} dilution of Moloney leukemia virus with a 50% spleen enlarging titer (SE_{50}) of 4.66 log units/ml. Beginning on the second day post inoculation and on each of Days 4, 8, 16, 32, 64, and 137, groups of ten infected mice were bled from the area of the brachial plexus. Spleens from the same animals were surgically removed, trimmed free from adipose and connective tissue, weighed, pooled, and minced into a cell suspension in 0.15 M cold potassium citrate.

In addition, groups of 16 newborn mice per dilution were each inoculated with 0.1 ml of the same stock virus diluted from 10^{-1} to 10^{-9}. Twenty-eight days later, these mice were killed. Their spleens were weighed, pooled according to virus dilution groups, and minced also into cell suspensions in cold potassium citrate.

Portions of these cell suspensions were washed and resuspended in saline at approximately 2-4 x 10^8 cells/ml. Several drops of these were placed on slides for immunofluorescent examination.

The remainder of the splenic material was thoroughly homogenized at 4°C in a tissue grinder as a 10% homogenate. This material was used as the extracted virus pool for the particular day of sampling. Log dilutions of this virus were inoculated subcutaneously at the dorsal junction of the forearm into each of 16-20 newborn mice per dilution.

Plasma from chilled, pooled blood of the infected mice was diluted and inoculated into newborn mice as described. The concentrated white blood-cell layer was washed in saline and used for the fluorescent antibody tests.

Titers of infectivity of the inoculated virus were obtained according to methods described by Chirigos et al. (1), Rowe (10), and Moloney (5). Twenty-eight days after inoculation all mice from each dilution group were killed and weighed. Their spleens were removed, trimmed of extraneous material, and weighed to the nearest mg. Animals weighing at least 10 gm and whose spleens weighed more than 150 mg contributed to the assay. These weight parameters are based on the rationale that mice weighing less than 10 gm were suspected of reovirus infections (11) since healthy 28-day-old BALB/c mice as produced at NIH normally weigh at least 10 gm. Selection of those mice whose spleens weighed more than 150 mg compensated for any contributory effects from secondary infections such as that caused by Bartonella-type organisms and the lactate dehydrogenase elevating agent (9). These weight limitations are those routinely used in the spleen-weight assay method for the Moloney leukemia virus (personal communication from Dr. Moloney).

After all mice from each viral dilution group had been tabulated, the mean log mg spleen weight of those which con-
the importance of this information is apparent when effective-
amount of virus associated with the disease state. For example,

Discussion

which infectious virus could be recovered. The immunoreac
tests might provide additional information by indicating the
virus infections (4, 8). A question is raised as to whether such
have been used to identify and characterize murine leukemia
of virus, recovered virus, and immunofluorescence (Chart 1).

The intervals between samplings, in this study, may have
tested to equate infectivity titers of virus with arbitrary units of immunofluorescence. The investiga-
tion was based on the assumption that increased viral replica-
tion, during pathogenesis of the disease in mice, would
enhance the fluorescent antibody reaction. An in vivo system for virally infected mice was chosen in the belief that such system could aid in the study of candidate viruses of possible etiologic significance to human leukemia and lymphoma. Although im-
munofluorescence as used in the present study detected as little
as 0.42 SE_{50} log units of infection, noninfectious particles may have reacted with the tagged antibody. It is likely also that the extraction procedures inactivated some infectious virus. However, it appears that the fluorescent antibody technic is indeed more sensitive in detecting viral antigens than are infectivity assays, in spite of the fact that it cannot distinguish biologically inert particles from infectious virions.

The present study attempted to equate infectivity titers of virus with arbitrary units of immunofluorescence. The investiga-
tion was based on the assumption that increased viral replica-
tion, during pathogenesis of the disease in mice, would
enhance the fluorescent antibody reaction. An in vivo system for virally infected mice was chosen in the belief that such system could aid in the study of candidate viruses of possible etiologic significance to human leukemia and lymphoma. Although im-
munofluorescence as used in the present study detected as little
as 0.42 SE_{50} log units of infection, noninfectious particles may have reacted with the tagged antibody. It is likely also that the extraction procedures inactivated some infectious virus. However, it appears that the fluorescent antibody technic is indeed more sensitive in detecting viral antigens than are infectivity assays, in spite of the fact that it cannot distinguish biologically inert particles from infectious virions.

The intervals between samplings, in this study, may have
tested to equate infectivity titers of virus with arbitrary units of immunofluorescence. The investiga-
tion was based on the assumption that increased viral replica-
tion, during pathogenesis of the disease in mice, would
enhance the fluorescent antibody reaction. An in vivo system for virally infected mice was chosen in the belief that such system could aid in the study of candidate viruses of possible etiologic significance to human leukemia and lymphoma. Although im-
munofluorescence as used in the present study detected as little
as 0.42 SE_{50} log units of infection, noninfectious particles may have reacted with the tagged antibody. It is likely also that the extraction procedures inactivated some infectious virus. However, it appears that the fluorescent antibody technic is indeed more sensitive in detecting viral antigens than are infectivity assays, in spite of the fact that it cannot distinguish biologically inert particles from infectious virions.
other. It appeared that immunofluorescence always preceded viral replication as measured by infectivity. This finding in an in vivo system confirms that of Osato et al. (6), who suggested that the synthesis of viral antigen occurred at a more rapid rate than did the maturation and release of virus in cell culture. Perhaps in this respect, Fink et al. (3) were able to correlate immunofluorescence with remissions and relapses of the disease state in patients with leukemia.

REFERENCES


Figs. 1-3. Photomicrographs were taken on Kodachrome film. Black and white reversed prints are shown here.

Fig. 1. Spleen cells from uninoculated mice. Direct staining with labeled rabbit anti-Moloney leukemia virus serum. × 1125.

Fig. 2. Spleen cells from mice inoculated with Moloney leukemia virus for 16 days. Indirect staining with unlabeled rabbit anti-Moloney leukemia virus serum followed with labeled goat anti-rabbit globulin serum. × 1125.

Fig. 3. Spleen cells from mice inoculated with Moloney leukemia virus for 16 days. Direct staining with labeled rabbit anti-Moloney leukemia virus serum. × 1350.
Immunofluorescence and Virus Recovery: Correlation in a Murine Leukemia System

Henry C. Orr, John B. Moloney, Joel I. Reisher, et al.


Updated version  Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/28/9/1793

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.