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Brief Communication

Immunofluorescence and Virus Recovery: Correlation in a Murine Leukemia System[1]

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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 4–8 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^{-6}. 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 × 10^3 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-

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tributed to the assay was calculated. The dilution of virus causing a 50% increase in spleen weights was determined both graphically and by the Reed-Muench method for calculating LD_{50} titers (7) and is referred to as the SE_{50} titer.

Immunofluorescent Procedures. Immune globulins from sera prepared in BALB/c mice and in rabbits according to procedures used by Fink (2) and Yosida (12) were labeled and used in the direct and indirect method of fluorescent antibody staining. A subjective method of scoring the intensity or degree of fluorescence from ± to 4+ was used for the direct method of staining. The indirect fluorescent antibody reaction was graded by choosing the reciprocal of the dilution of unlabeled rabbit antiserum which gave a 1+ reading.

Results

The two methods of immunofluorescence appeared distinct (Figs. 1–3). Apparently, the direct fluorescent antibody method detected an antigen different from that detected by the indirect procedure. In contrast, the location of at least one of the antigens was such that it could be located by only one of the immunofluorescent procedures. By neither method was immunofluorescence demonstrable the first two days after virus inoculation. An equivocal fluorescent reaction occurred four days after inoculation when the direct method produced fluorescence in the nucleolar area of the cells. During the pathogenesis of the disease, this fluorescence spread first to the entire nuclear area and later throughout the entire cell. The same pattern was exhibited by both labeled antisera.

The indirect method of fluorescent antibody staining resulted in a different pattern. From the 8th day after virus inoculation, the immunoreaction spread from the cell surface through the cytoplasm to the perinuclear area. Thus, the pattern of fluorescence appeared to be variable with regard to the method of staining.

No infectious virus could be recovered from infected mice before the 16th day, when 1.09 SE_{50} log units of virus were recovered from the plasma and 0.42 SE_{50} log units of virus were extracted from spleens of inoculated mice. Although different quantities of virus were recovered from the two tissues, there were no discernible differences between them in the immunofluorescent test.

In addition, there was little correlation between concentration of recovered virus and immunofluorescence. There was greater correlation between initiating dose of virus and host response to the infection. During pathogenesis of Moloney viral leukemia, fluorescence seemed to precede in time the point at which infectious virus could be recovered. The immunoreaction rapidly increased to a plateau; thus, it was difficult to establish a linear stepwise relationship between initiating dose of virus, recovered virus, and immunofluorescence (Chart 1).

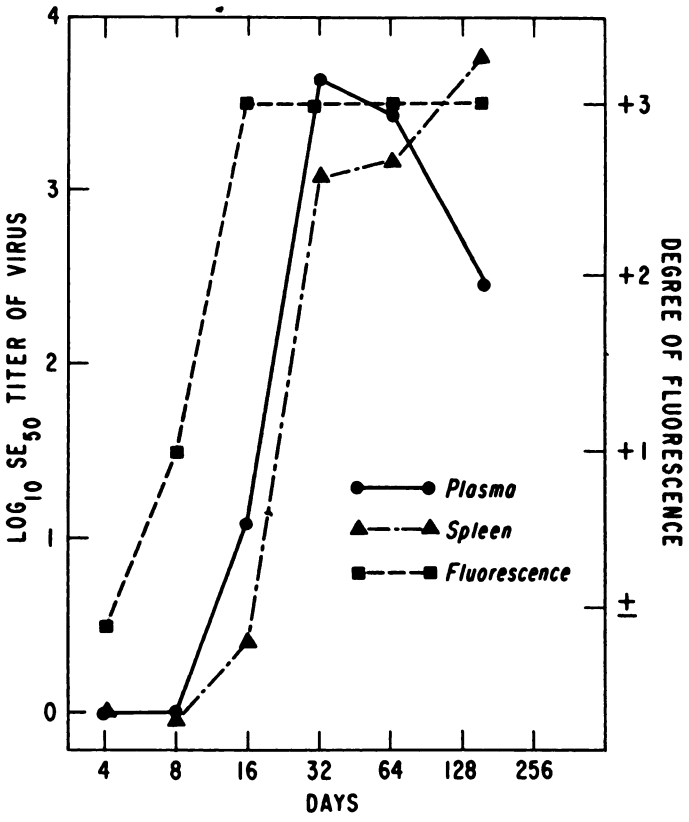

Chart 1. Correlation of recoverable virus and immunofluorescence (direct method) during pathogenesis of Moloney leukemia virus infection in mice.

Discussion

Various diagnostic procedures, including immunofluorescence, have been used to identify and characterize murine leukemia virus infections (4, 8). A question is raised as to whether such tests might provide additional information by indicating the amount of virus associated with the disease state. For example, the importance of this information is apparent when effectiveness of therapy in these viral diseases is to be evaluated. This consideration alone has justified the intensive work involved in relating serologic test values to known quantities of virus in defined systems.

The present study attempted to equate infectivity titers of virus with arbitrary units of immunofluorescence. The investigation was based on the assumption that increased viral replication, during pathogenesis of the disease in mice, would enhance the fluorescent antibody reaction. An *in vivo* system for viral replication 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 immunofluorescence 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 been too large to establish definitive sequential correlation of immunofluorescence with virus titers. For example, only three stages of fluorescence and two stages of viral replication were noted. Moreover, these stages were not in agreement with each

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.

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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.