PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 155, 137-141 (1977)

Detection of Tumor-Specific Antigen and Antibody in Kidneys of Neuroblastoma-Bearing Mice (39760)

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The mouse C-1300 tumor arose spontaneously in the body cavity of an albino male A/Jax mouse and was tentatively diagnosed as neuroblastoma (1). The biochemical and morphologic characteristics of the mouse neuroblastoma have been studied extensively (2). From an immunologic standpoint, some surface membrane antigens on the mouse neuroblastoma have been characterized (3). In addition, there have been reports of tumor-specific antibodies eluted from the C-1300 tumor (4) and evidence of tumor-specific antibodies and immune complexes in the sera of tumor-bearing mice (4–6). The kidney is a frequent site for deposition of circulating immune reactants which may give rise to glomerular inflammation. Accordingly, in the present study we examined the kidneys of mice bearing neuroblastoma for evidence of glomerular inflammation and immune deposits. Our findings demonstrate a mesangial proliferative reaction with evidence of immunoglobulin and complement deposition in the renal glomerulus distributed in a mesangial pattern. We further demonstrate that these deposits consist of immune complexes composed of neuroblastoma antigen and specific antibody.

Methods. A/Jax male mice 10 weeks of age (R. B. Jackson Laboratories, Bar Harbor, Maine) were used. The C-1300 neuroblastoma growing in A/Jax male mice was obtained from Dr. Kedar Prasad. Techniques employed for preparing tumor cell suspensions, for injecting tumor cells, and for irradiating mice have been described (4, 7).

An antisera specific for neuroblastoma was prepared by injecting 3-kg New Zealand white rabbits (Mile High Rabbitry, Golden, Colo.) with fresh neuroblastoma cells (3×10^8) incorporated in complete Freund's adjuvant and boosting them 5 weeks later by iv injection of 10^8 neuroblastoma cells. Antisera were harvested 16 days later and absorbed with normal A/Jax mouse serum, kidney, liver, and brain as previously described (4).

Eluates were obtained from neuroblastoma cells and from 20 kidneys of neuroblastoma-bearing mice by previously described methods (4, 8–10.) The eluates, examined by immunoelectrophoresis using heterologous antisera to mouse IgG, whole sera, and mouse serum albumin, were found to contain only mouse IgG.

Immunoglobulins obtained from eluates and antisera were conjugated to FITC and employed to stain tumor or kidney sections by previously described methods (8–10).

Experiments, results, and discussion. Viable neuroblastoma cells, 10^6, were injected intradermally into 14 A/Jax male mice 10 weeks of age. Seven days later, tumors were palpable in all animals. The mice were sacrificed on Day 10. Kidney sections were prepared and stained with hematoxylin and eosin and periodic acid Schiff and 2-μm sections were prepared for fluorescent microscopy employing separate rabbit antisera to mouse immunoglobulins and complement (Miles Laboratory, Kankakee, Ill.). All kidney sections examined showed moderate focal and segmental glomerular mesangial proliferation. By fluorescent microscopy, all kidneys showed diffuse and segmental deposition of mouse immunoglobulin and complement in the renal glomerulus. A control group of seven A/jax mice was sublethally irradiated with 500 R, injected intradermally with 10^6 tumor cells at the same time as experimental mice, and also sacrificed 10

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ISSN 0037-9727

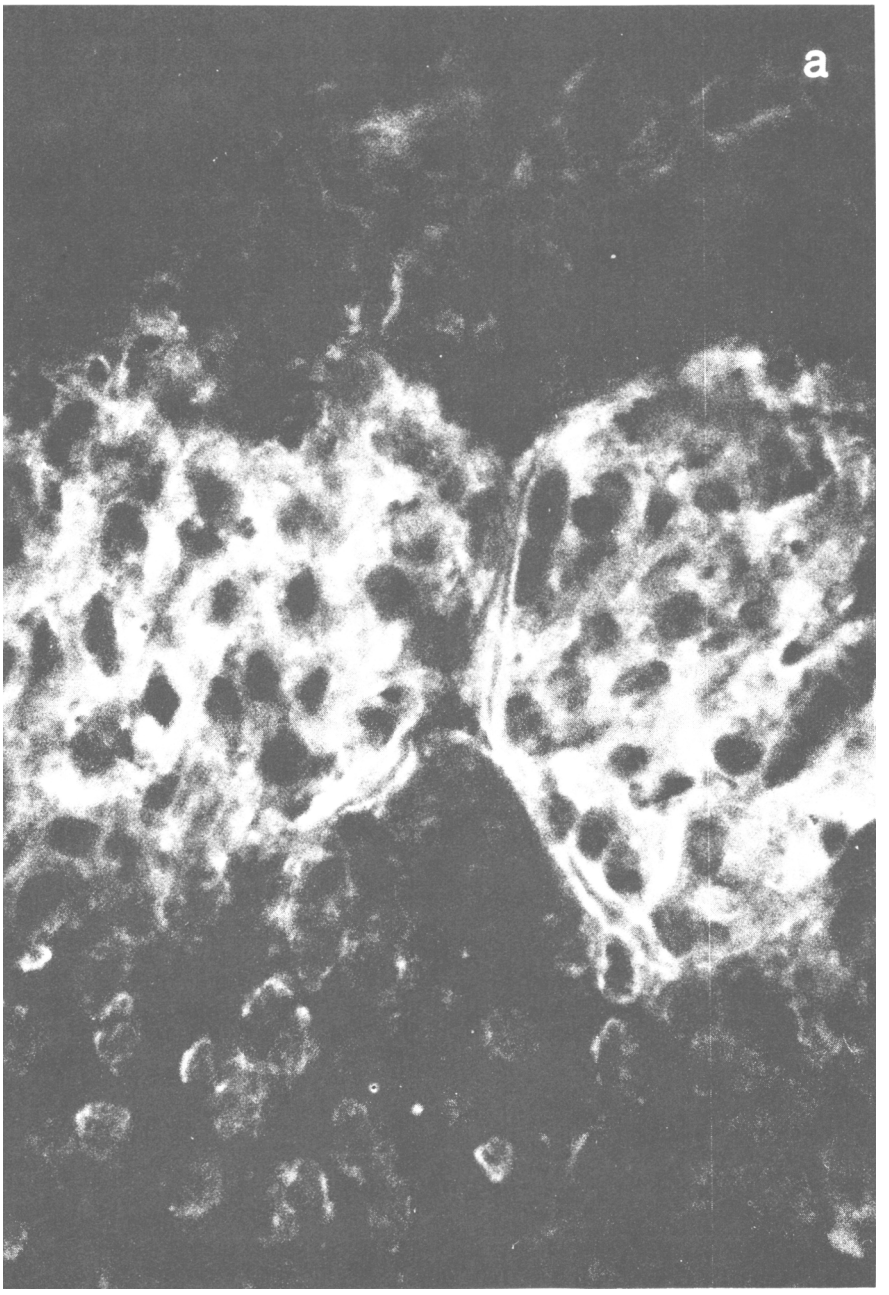

FIG. 1. Immunofluorescent studies of kidneys from neuroblastoma-bearing mice 10 days after tumor inoculation are depicted. (a) Diffuse mesangial deposition of neuroblastoma antigen using rabbit antisera to neuroblastoma. (b) Positive staining of neuroblastoma cells with an eluate obtained from kidneys of neuroblastoma-bearing mice 10 days after tumor inoculation.

days later. All of these animals demonstrated palpable tumors by Day 6. However, in contrast to the nonirradiated group, sections of kidneys from these animals failed to demonstrate mesangial proliferation or deposition of mouse immunoglobulins by immunofluorescence.

A heterologous antisera specific for neu-

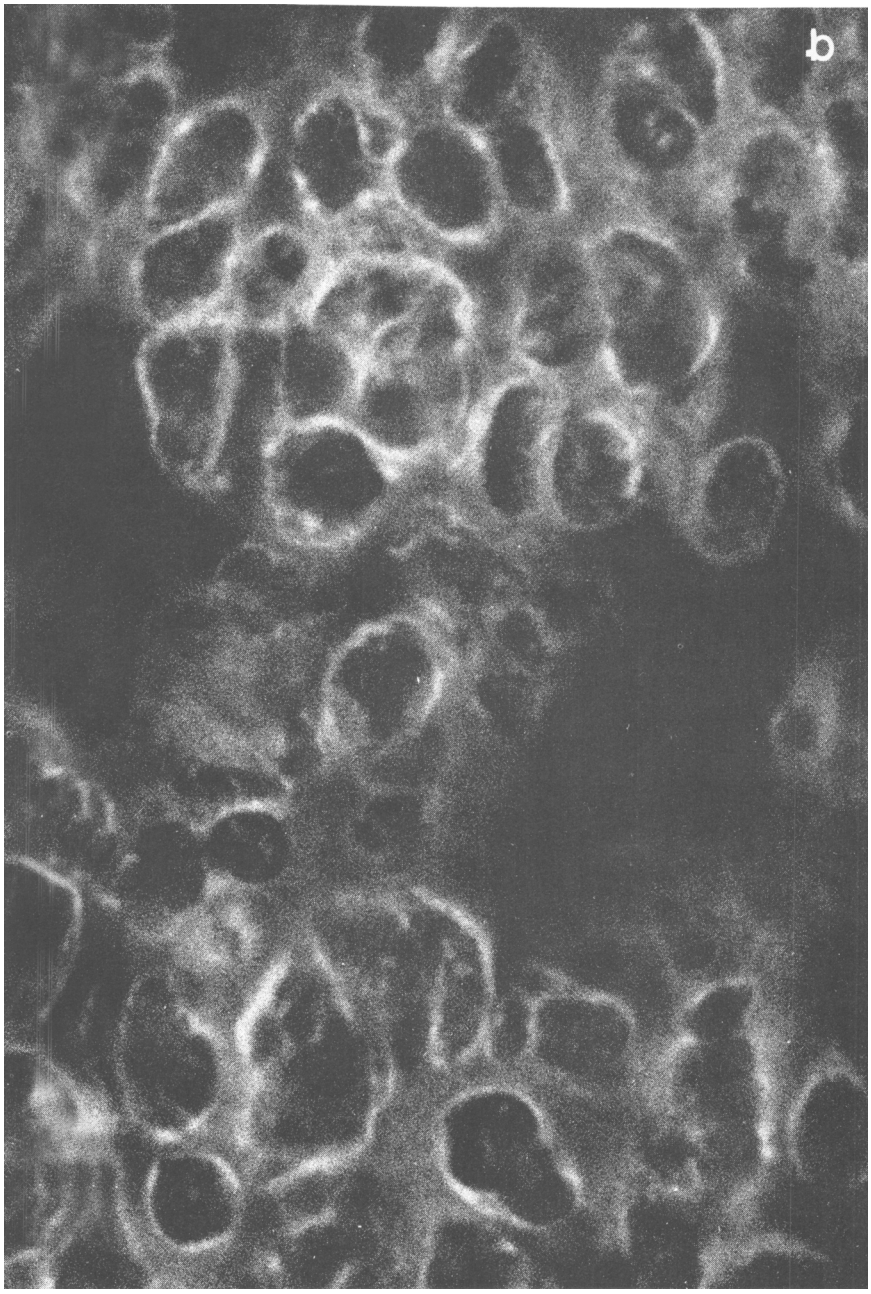

FIG. 1—*continued*

roblastoma was prepared and the immunoglobulin fraction was isolated, conjugated with FITC as described in Methods, and incubated with a fresh preparation of tumor cells. A homogeneous pattern of staining of tumor cells was observed. The same heterologous antisera was employed to stain glomeruli of seven tumor-bearing mice 10 days after tumor inoculation for the presence of neuroblastoma antigen. In all mice studied, neuroblastoma antigen was deposited in glomeruli in a mesangial distribution (Fig. 1a). The staining of both tumor cells and glomeruli of neuroblastoma-bearing mice was abol-

ished by absorption of antisera with viable neuroblastoma cells.

An eluate was obtained from 20 kidneys of nonirradiated neuroblastoma-bearing mice 10 days after tumor inoculation. The eluate was conjugated with FITC and incubated with viable neuroblastoma tumor cells which resulted in diffuse staining (Fig. 1b) that was abolished by absorption of eluate with viable neuroblastoma cells.

An eluate in which IgG antibodies were identified by immunoelectrophoresis was obtained from purified neuroblastoma cells. These antibodies were conjugated with FITC and incubated with sections of kidneys from seven nonirradiated neuroblastoma-bearing animals 10 days after tumor inoculation. All kidneys studied demonstrated glomerular fluorescence distributed in a focal, segmental mesangial pattern. This reaction was abolished by absorption of eluate with viable neuroblastoma cells.

These data form a basis for understanding the nature of the mesangial proliferative reaction observed in kidneys of neuroblastoma-bearing mice shortly after tumor inoculation. Further, these findings suggest that mouse neuroblastoma antigen is deposited in glomeruli of tumor-bearing animals together with specific immunoglobulins. The immune complexes appear to be the consequence of a specific immune response since no immune deposits were observed in kidneys of A/Jax mice that were sublethally irradiated and similarly injected with neuroblastoma cells. Neuroblastoma-specific antigen was demonstrated in glomeruli of tumor-bearing mice with a heterologous antisera specific for neuroblastoma; this glomerular staining was completely abolished by absorption of antisera with neuroblastoma cells. An eluate obtained from the neuroblastoma cells also stained the glomeruli of tumor-bearing mice and an eluate obtained from the kidneys of tumor-bearing animals stained the tumor. Staining by both eluates was abolished by absorption with viable neuroblastoma cells. These data would then strongly confirm in detail the earlier studies of Oldstone (5) demonstrating that the immune deposits in glomeruli of neuroblastoma-bearing mice consist of neuroblastoma-specific antigen complexed with specific antibody.

While the association of glomerulonephritis with neoplastic processes is recognized (10), in only a few instances have tumor-specific antigens been identified in glomeruli of tumor-bearing hosts (11–13). This study thus lends further support for the role of tumor-specific immune reactants as the basis of glomerular inflammation in tumor-bearing hosts. The kidney may be a fruitful source and for isolation and characterization of tumor-specific immune deposits which are trapped in glomeruli shortly after tumor inoculation. These immune reactants may represent enhancing or blocking factors which are considered by some to be immune complexes (14). Present studies are aimed at isolating these complexes and assessing their immunobiological function.

Summary. Immune deposits were observed in the glomerular mesangium of neuroblastoma-bearing mice which was associated with a mesangial proliferative reaction. Employing specific heterologous antisera and an eluate obtained from neuroblastoma cells, neuroblastoma antigen was identified in kidneys of tumor-bearing mice. Immunoglobulin-containing eluates from kidneys of neuroblastoma-bearing mice were shown to specifically react with viable neuroblastoma cells. These data suggest that the immune deposits noted in kidneys of neuroblastoma-bearing mice are composed of neuroblastoma antigen together with specific antibody and lend further support to the role of tumor-specific immune reactants as causative factors in the pathogenesis of glomerular inflammatory reactions in neuroblastoma-bearing mice.

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Received January 13, 1977. P.S.E.B.M. 1977, Vol. 155.