Immunity to Cell Surface Antigens and Immune Complex
Glomerulonephritis in Hamsters Bearing Human
Epithelial Tumors

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ABSTRACT

A glomerulopathy in hamsters bearing human epithelial tumors is described. The tumors resulted from the s.c. implantation of human heteroploid cells (MA16O) derived originally from a benign prostatic adenoma. The renal immunomorphological and ultrastructural changes include expansion of mesangial matrix and cellularity, electron-dense mesangial deposits, and mesangial deposits of immunoglobulin G and C3. Renal eluates obtained from the tumor-bearing animals contained substantial amounts of immunoglobulins. Our immunopathological studies demonstrated that virtually all of the eluate immunoglobulin G was tumor-specific antibody directed exclusively against surface determinants of the MA16O cells. There was no demonstrable activity against cytoplasmic or nuclear constituents of the MA16O cells or against certain hamster antigenic determinants. The antibody in the eluate could be removed by repetitive absorptions with viable MA16O cells but not with similar absorptions with either normal hamster BHK21 fibroblasts, human heteroploid HeLa cells, or MA16O hamster cells.

Our data support the concept of a specific nephritogenic immunopathological process occurring as a result of immunity to tumor-specific surface determinants.

INTRODUCTION

In previous communications, we reported the growth of human heteroploid cells derived from a prostatic adenoma (MA16O) in normal golden Syrian hamsters (17). Twenty to 30% of animals receiving 10^7 cells s.c. on the second day of life sustained progressive tumor growth for variable but finite periods of time extending to 7 months after inoculation. Marked increases in serum immunoglobulin concentration were noted in animals with tumors (15). Some of the immunoglobulin was specific anti-tumor cell surface antibody (16).

Because preliminary morphological observations suggested that the tumor-bearing animals developed glomerulonephritis, we performed sequential light and electron microscopic, immunohistological and other immunopathological studies on 14 hamsters with tumors at times ranging from 4 to 17 weeks postinoculation. Fourteen control littermates were similarly examined. In this communication, we report the observation of immune complex glomerulonephritis and demonstrate the participation of tumor-specific cell surface antigens in the major underlying immunopathogenic mechanism.

MATERIALS AND METHODS

Cell Cultivation, Heterotransplantation, and Animal Harvest Techniques. Cell culture and heterotransplantation techniques have been described previously (17). In short, MA16O cells were recovered from suspension culture, washed, and then inoculated s.c. into the dorsocaudal area of 2-day-old golden Syrian hamsters.

The animals with tumors were sacrificed by exsanguination. Individual animals were harvested sequentially at weekly intervals beginning at 4 weeks and extending to 17 weeks after tumor implantation. At each time, a control littermate was similarly harvested. Complete autopsies were performed on all animals. In addition, portions of kidney were prepared for immunofluorescence, electron microscopy, and elution studies (see below).

Light Microscopy. Tissue for light microscopy was fixed in Van de Grift's solution, embedded in paraffin, and sectioned 2 to 3 μm thick. The following histochemical stains were used: hematoxylin and eosin, periodic acid-Schiff, Masson's trichrome, and periodic acid-Schiff methenamine silver with a Masson trichrome counterstain. At least 100 glomeruli were examined from each harvested animal.

Electron Microscopy. Portions of hamster kidneys were fixed in phosphate-buffered paraformaldehyde, postfixed in 2% osmium tetroxide, dehydrated, and embedded in epoxy resin. Ultrathin sections were stained with lead citrate and uranyl acetate and viewed with a Zeiss EM9 and a Hitachi HU-IIC electron microscope. A minimum of 5 glomeruli from each kidney specimen were examined ultrastructurally.

Immunofluorescent Staining of Kidneys. Indirect immunofluorescent staining procedures were those employed routinely in this laboratory and have been published previously (17). Cryostat kidney sections were stained with FITC-goat anti-hamster IgG (Cappell Laboratories, Downingtown, Pa.), FITC rabbit anti-hamster C3 (obtained through Dr. David Porter, University of California at Los Angeles, Los Angeles, Calif.), and FITC goat anti-hamster albumin. Immunological specificity of the antisera was confirmed by immunoelectrophoresis. Each antiserum showed a single precipitin line with whole hamster serum.

Immunoglobulin Elution from Kidneys. Portions of kidneys from 10 tumor-bearing animals were pooled, homogenized, and eluted according to the method of Lambert and Dixon (7). A pool of kidneys from 10 control littermates was eluted and absorbed, and the immunoglobulin was eluted (see below).

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similarly eluted. The eluate proteins were concentrated by pressure dialysis, quantitated by the method of Lowry et al. (9), and characterized by cellulose acetate electrophoresis and immunoelectrophoresis with both anti-whole hamster serum and anti-hamster IgG. Eluate IgG was quantitated by radial immunodiffusion according to the method of Mancini et al. (10).

**Indirect Membrane Immunofluorescence against MA160 Tumor Cells.** Indirect membrane immunofluorescence tests were performed to determine whether the eluate contained antibody against surface constituents of the MA160 cells. Procedures for indirect membrane immunofluorescence have been published previously (16). In brief, freshly harvested and washed viable MA160 cells were incubated with the renal eluate, followed by a similar incubation with fluoresceininated goat anti-hamster immunoglobulin conjugate. Controls included normal hamster sera plus conjugate and conjugate alone.

**Renal Eluate Absorptions.** One ml of eluate was absorbed with approximately 1 x 10^6 viable cells harvested from tissue culture. Absorptions were performed overnight at 4°.

**RESULTS**

**Light Microscopy.** The kidneys from tumor-bearing animals harvested from 4 to 6 weeks after tumor inoculation were essentially normal. Beginning at 7 weeks, histopathological changes were observed. At this time, rare glomeruli (less than 5%) showed segmental increases of periodic acid-Schiff-positive mesangial matrix and mesangial cellularity. As tumor growth progressed, a greater percentage of glomeruli became involved. At 10 to 13 weeks, 50% of the glomeruli showed this segmental mesangial expansion. Neutrophils were identified in occasional glomeruli, and rare capillary loops were occluded by fibrin. These changes were most severe at 15 to 17 weeks, when 95 to 100% of the glomeruli manifested diffuse increases in mesangial cells and matrix (Fig. 1) with segmental synechiae and adhesions to Bowman's capsule. Capillary loops were consistently of normal thickness and remained patent until about 15 weeks, when swelling and vacuolization of endothelial cells became manifest. This endothelial cell alteration severely compromised the capillary lumens.

**Electron Microscopy.** Focal foot process fusion was the first ultrastructural abnormality recognized, occurring as early as 5 weeks after tumor inoculation. Confirming the light microscope observations, increases in mesangial matrix and cellularity were observed as early as 7 weeks. These changes became more generalized and intense with increasing time after tumor implantation. Beginning at 7 weeks, electron-dense deposits could be identified in the mesangial regions. These deposits increased in size and number throughout the period of observation (Fig. 2). Subepithelial and intramembranous deposits along the peripheral capillary wall were noted at 11 weeks after tumor inoculation (Fig. 3) but were not observed at other times. Beginning at 15 weeks, marked swelling and vacuolization of the endothelial cells were observed, and this was most intense in the last animal studied at 17 weeks after tumor inoculation (Fig. 4).

**Immunofluorescence of Kidneys.** Generalized granular deposits of IgG and C'3 were noted in the glomerular mesangium of all tumor-bearing animals (Fig. 5). Absorption of the FITC anti-hamster IgG and the FITC anti-hamster C'3 with purified hamster IgG and C'3, respectively, completely abolished the positive immunofluorescent staining. In addition, the fluorescence was blocked by prior incubation of the sections with unlabeled anti-hamster IgG and anti-hamster C'3. No positive staining reactions were seen with the FITC anti-hamster albumin. None of the control kidneys showed deposition of IgG of C'3.

**Antibody Specificity or Immunoglobulin Eluted from Kidneys.** Four hundred seventy μg of immunoglobulin per g of kidney were eluted from the pool of kidneys obtained from the animals with tumors (Table 1). The immunoglobulin was exclusively IgG. There was no demonstrable immunoglobulin in the control eluate.

The renal eluate from the tumor-bearing animals was absorbed with viable MA160 cells until it no longer elicited positive membrane-staining reactions. Three absorptions were necessary to remove specific MA160 cell membrane-binding antibody completely. Approximately 20 μg of IgG per g of kidney remained in the eluate after this absorption, indicating that greater than 95% of the immunoglobulin had been absorbed by the surface constituents of the MA160 cells. Three similar absorptions were performed with each of 3 cell lines: normal human diploid skin fibroblasts or human heteroploid cells (HeLa). Furthermore, the eluate did not contain detectable antibody against normal hamster lymphocytes or erythrocytes.

The eluate reacted strongly with the MA160 cells, showing generalized, intense patchy membrane staining (Fig. 6). There was no activity against cytoplasmic or nuclear constituents of the MA160 cells as determined using acetone-fixed MA160 cells in indirect immunofluorescence, and essentially no activity was demonstrable against either viable human diploid skin fibroblasts or human heteroploid cells (HeLa). Furthermore, the eluate did not contain detectable antibody against normal hamster lymphocytes or erythrocytes.

The eluate was assayed for activity against the three cell lines. Serum from normal hamsters was included as a positive control. The eluate showed no activity against any of the cell lines or against normal hamster lymphocytes. The eluate was centrifuged to remove any precipitated material. The supernatant was subjected to immunofluorescence with the three cell lines, and the results were consistent with the results obtained with the uncentrifuged eluate. Absorption of the eluate with normal hamster lymphocytes removed antibody activity against this cell type. Absorption of the eluate with MA160 cells removed antibody activity against these cells. Absorption of the eluate with human diploid skin fibroblasts or HeLa cells removed antibody activity against these cell lines. Absorption of the eluate with human heteroploid HeLa cells and hamster BHK21 cells removed antibody activity against these cell lines.

**Table 1**

<table>
<thead>
<tr>
<th>Eluates from</th>
<th>Immunoglobulin in eluates</th>
<th>Antibody specificity of eluate immunoglobulin</th>
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<tbody>
<tr>
<td>Ten tumor-bearing hamsters (mean 24-hr urine protein, 19.4 mg)</td>
<td>470 μg of IgG/g kidney</td>
<td>+ for surface determinants of MA160 cells; – for cytoplasmic constituents of MA160 cells, DNA deoxyribonucleoprotein hamster lymphocytes, hamster RBC</td>
</tr>
<tr>
<td>Ten control littermate hamsters (mean 24-hr urine protein, 1.0 mg)</td>
<td>No demonstrable immunoglobulin</td>
<td>No activity demonstrable</td>
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Urine protein was quantitated by the sulfosalicylic acid technique (6).
DISCUSSION

Antigen-antibody complex-mediated glomerulopathies have been reported in tumor-bearing experimental animals and humans. AKR mice have a high incidence of glomerular disease secondary to the deposition of immune complexes containing Gross viral antigens and antibody directed against these antigens (12). Furthermore, RII mice with virus-induced mammary tumors have glomerular disease with immune complexes containing tumor-specific viral antigen and antibody (14). In humans, an association of antigen-antibody complex glomerulopathies and carcinoma has been described (4, 13). In some instances, these complexes have been shown to contain specific antitumor antibody (2, 8). Circulating immune complexes have been detected in patients with various forms of leukemia, and their presence has been correlated with an unfavorable prognosis (1).

The data we have presented in this report support the concept of a specific nephritogenic immunopathological process occurring as a result of immunity to cell-surface antigens. Whereas virtually all of the renal eluate IgG was specific antibody against MA160 cell surface antigens, only a small fraction of the total serum IgG complement was specific MA160 surface antigen-directed antibody (16). A saturation of antigen-binding sites in the form of antigen-antibody complexes could explain the paucity of circulating free antibody. This hypothesis is supported by our present experimental data.

An experimental system analogous to our system has been observed in mice with chronic allogeneic disease (5). Chronic allogeneic disease constitutes a spectrum of immunopathology secondary to the chronic graft versus host reaction (3, 5). In these animals, glomerulonephritis develops, and antibody directed against surface constituents of the donor lymphocytes is demonstrable in the glomerular eluates of these animals (5). What proportion of the glomerular immune complexes is composed of such complexes, however, has not been established. Circulating immune complexes, as well as granular deposits of IgG and complement, have been detected in mice bearing syngeneic neoplasms (11). IgG eluted from the kidneys demonstrated specificity against tumor cell surface determinants (11). However, these animals exhibited no histological or clinical evidence of renal disease.

The exclusive participation of tumor cell surface determinants in the pathogenesis of this immune complex glomerulonephritis renders this experimental model unique. Experiments are currently in progress to characterize further the surface antigens of the MA160 cells.

In summary, we have described generalized glomerulonephritis in hamsters bearing human epithelial tumors. Immunopathological studies established an immune complex etiology for the renal disease. Renal eluates from the tumor-bearing animals contained substantial amounts of tumor-specific IgG, virtually all of which was directed against surface constituents of MA160 tumor cells.

REFERENCES

Fig. 1. Hamster 15 weeks after tumor transplantation. Glomerulus shows increase in mesangial matrix and cellularity. H & E, × 750.

Fig. 2. Hamster 12 weeks after tumor transplantation. This electron micrograph shows an enlarged and hypercellular mesangial area of the glomerulus with numerous electron-dense deposits buried in the mesangial matrix. Uranyl acetate and lead citrate, × 14,000.
Fig. 3. Hamster at 11 weeks after tumor transplantation. The peripheral capillary wall demonstrates several subepithelial and intramembranous electron-dense deposits. Note also the swelling and fusion of the epithelial cell foot processes. Uranyl acetate and lead citrate, × 20,000.

Fig. 4. Hamster 17 weeks after tumor transplantation. Increases in mesangial matrix and cellularity with numerous mesangial electron-dense deposits can be appreciated. The most significant change, however, is marked swelling and ballooning degeneration of the endothelial cells, which completely occlude the capillary lumens in some areas. Uranyl acetate and lead citrate, × 5,500.
Fig. 5. Immunofluorescent photomicrograph demonstrating granular deposits of IgG in a mesangial distribution. × 725.

Fig. 6. Generalized patchy membrane immunofluorescence of viable MA160 cells obtained after 30 min incubation with eluate from tumor-bearing animals followed by FITC anti-hamster immunoglobulin for an additional 30 min. The cells were washed 3 times in phosphate-buffered saline, pH 7.2, after both the eluate incubation and the conjugate incubation. After the final wash, the cells were mounted in a 50% glycerol-phosphate-buffered saline solution. × 800.
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