RENAL DEPOSITION OF SOLUBLE IMMUNE COMPLEXES IN MICE BEARING B-16 MELANOMA

CHARACTERIZATION OF COMPLEXES AND RELATIONSHIP TO TUMOR PROGRESS*

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There are indications from scattered case reports in the medical literature of an association between the nephrotic syndrome and a variety of extrarenal malignancies in humans (1–10). Histologic examination of renal biopsies often revealed a membranous or membranoproliferative glomerulonephritis in these patients (1, 5, 8–10). Electron microscopic or fluorescent antibody studies indicated that the histologic alterations were associated with glomerular deposition of immune complexes. Furthermore, in one case, immunoglobulin eluted from the kidney was reactive against autologous tumor cell membranes (8).

In the course of examining tissues for microscopic metastases in C57BL/6J mice bearing a strain-specific melanoma (B-16), we routinely found histologic alterations of the renal glomeruli characterized by a mild proliferative glomerulonephritis (Fig. 1). These mice had slightly elevated urine protein levels, but they were not significantly above normal limits. However, the histologic alterations of the kidneys were striking and were not due to metastatic infiltration or impairment of the renal blood supply by the tumor. It was of interest to determine in what way this asymptomatic renal damage was related to the presence and progress of the tumor; especially, whether it reflected the generation and deposition of soluble complexes of tumor antigen and antitumor antibody. Such complexes have been implicated in the blockade of cell-mediated tumor rejection in other systems (11, 12).

Materials and Methods

The B-16 melanoma is a transplantable, strain-specific tumor of C57BL/6 mice, which was spontaneous in origin. Subcutaneous inoculation of $1 \times 10^6$ cells produces a progressive, local, lethal growth in at least 70% of mice in our experience. The mice in this study were male C57BL/6 obtained from the Jackson Laboratories, Bar Harbor, Maine.

Suspensions of tumor cells used in all assays and for in vivo immunization or challenge

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Fig. 1. (A) Kidney from normal 16-wk old C57BL/6J male mouse. (B) Kidney from 16-wk old tumor-progressor C57BL/6J male mouse showing prominent hypercellularity and increased mesangial matrix. × 400. Hematoxylin and eosin-stained 3 μm sections.
were derived from nontrypsinized B-16 cells maintained in vitro in RPMI-1640 medium containing 15% heat-inactivated fetal calf serum, penicillin-streptomycin (50 U/ml), and tylocine (anti-PPLO) at 37°C in 5% CO₂ atmosphere.

Detection of Renal Immunoglobulin Deposits.—Kidneys obtained from animals killed by cervical dislocation were washed in cold saline, frozen in a dry-ice acetone bath, and stored at −70°C until sectioned. 6 μ cryostat sections were fixed and stained as previously described (13). Fluorescein-conjugated, monospecific antimouse IgG, IgM, and IgA were obtained from the Meloy Laboratory, Springfield, Va. Fluorescence was examined with a Zeiss Photomicroscope (Carl Zeiss, Inc., New York) equipped with an HBO 200 mercury vapor lamp, darkfield condenser, UG-1 exciter filter, and 47/65 barrier filter.

Histology.—Histologic examination of kidneys and tumors was done on formalin-fixed, paraffin-embedded tissues. The sections were cut at 3 μ and stained with hematoxylin and eosin.

In Vitro Assays for Cellular and Humoral Reactivity.—Plasma and peripheral blood leukocytes from tumor-sensitized, tumor-progressor, and tumor-rejector mice were obtained at intervals by bleeding from the retroorbital sinus under light ether anesthesia using sterile heparinized capillary tubes. 0.4–0.5 ml of blood could be safely taken from individual animals at 10-day intervals by this method. The blood was centrifuged and the plasma removed. Erythrocytes in the cell pellet were lysed by incubation with Tris-ammonium chloride for 10 min at 37°C. The leukocytes were washed four times with Hank’s balanced salt solution to remove residual serum and lysing solution. The cell number and viability were determined by hemocytometer counting in an eosin Y solution (14). Wright’s stained smears were prepared on random samples to determine the differential cell count. Leukocyte preparations showed greater than 95% viability after lysing and washing, and were found to consist of 97–100% lymphocytes. A yield of 1–2 × 10⁶ viable lymphocytes could be obtained from 0.4–0.5 ml of blood by this technique. Each animal’s plasma and leukocyte preparations were tested individually.

In vitro cytotoxic activity of the peripheral blood lymphocytes (PBL) was determined by admixture of 2 × 10⁵ PBL with 1 × 10⁵ [3H]TdR-labeled (1.9 Ci/mmol, Schwartz-Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.) B-16 cells (2:1 PBL: target cell ratio). The mixture was incubated at 37°C for 72 h in RPMI-1640 medium containing 5% fetal calf serum and the antibiotics described previously. During the incubation time the viable B-16 cells adhere to the glass culture tube. The number of viable tumor cells remaining at the end of the incubation period was estimated by the retention of [3H]TdR in trichloroacetic acid-precipitable material in adherent tumor cells. Isotope counting was done in a liquid scintillation system. Antibody cytotoxicity was determined in a similar fashion. The noncomplement inactivated plasma was diluted 1:10 in culture medium and the labeled tumor cells (1 × 10⁶) were suspended in 0.25 ml of the diluted serum and incubated for 1 h at 37°C. The volume was then brought up to 1.5 ml by addition of culture medium and the incubation was continued for 72 h. The viable target cells remaining were estimated by determining isotope retention as described above. The “blocking activity” of plasma was determined by first incubating the target cells with plasma as described for 1 h and then adding the PBL (2 × 10⁵) in a volume of 1.25 ml. Cytotoxicity was measured at 72 h as before.

The relative titers of anti-B-16 membrane reactivity of the plasma were determined using a mixed hemadsorption assay. B-16 cells were allowed to adhere to the surface of the wells of Trans-Type leukocyte-typing slides (Hyland Div., Travenol Laboratories, Inc., Costa Mesa, Calif.) and were subsequently incubated for 30 min at room temperature with dilutions of complement-inactivated mouse plasma. The monolayers were washed gently in phosphate-buffered saline (PBS) and two drops of a 0.5% suspension of doubly sensitized (mouse anti-

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1 Abbreviations used in this paper: GBM, glomerular basement membrane; MNL, mononuclear leukocyte; PBL, peripheral blood lymphocytes; PBS, phosphate-buffered saline.
SRBC/rabbit antimouse Ig) sheep red blood cells (SRBC) were added. After an additional 30-min incubation at room temperature the monolayers were again gently washed by dipping in PBS and were read for adherence of SRBC. The titer was taken as the last dilution of plasma showing significant SRBC adherence to target cells.

**Elution of Renal Immunoglobulin.**—Immunoglobulin was eluted from the kidneys of 9-wk old tumor-progressor mice by acid citrate or chaotropic ions using the method described by Linder, et al. (15). The eluates are designated citrate eluate and KI eluate, respectively.

**Detection of Tumor Antigen in Plasma.**—Individual plasma pools were prepared from five groups of mice. The first pool was derived from mice sensitized with soluble B-16 antigen but not challenged with tumor. These mice had demonstrable antitumor antibody. The second and third pools were derived from tumor-progressor mice at day 10 and 20 post-challenge, respectively. The day 10 plasma had no anti-B-16 antibody while the day 20 plasma did show significant antibody activity. The fourth pool was derived from day 20 plasma of animals which subsequently spontaneously rejected a tumor challenge of $1 \times 10^6$ cells and had no demonstrable antibody activity. The fifth pool was derived from control (nonsensitized, nonchallenged) mice. Each whole plasma pool was reacted in Ouchterlony double diffusion plates with rabbit antiserum against soluble B-16 antigen prepared as previously described. In addition, a 3-ml sample of each plasma pool was applied to a Sephadex G-150 column (2.5 × 45 cm) and eluted with PBS pH 7.4 at a flow rate of 10 ml/h. The excluded protein peak from each sample was concentrated by negative pressure dialysis and dialyzed against pH 3.0 saline to split antigen-antibody complexes. The samples were then dialyzed through an XM-50 Amicon membrane (Amicon Corp., Lexington, Mass.) with 10 vol of pH 3.0 saline. The dialysate, containing materials of less than 50,000 mol wt, was concentrated to one-half the original serum volume, dialyzed against pH 7.4 saline, and tested for the presence of B-16 specific antigen in double diffusion against rabbit anti-B-16SA antiserum.

**RESULTS**

The intensity of immunoglobulin deposition in representative tumor-progressor and age-matched control mice is shown in Table 1. IgG, IgM, and IgA deposits in the kidneys of tumor-progressor mice were always greater than in age-matched controls, although age-related, spontaneous complex deposition involving all three immunoglobulin classes did occur. IgG deposits showed the most consistent and most profound elevation in the tumor-progressor mice and always involved the glomerular basement membrane (GBM) as well as the mesangium; whereas in control mice the deposits, when present, were confined to the mesangium. The immunofluorescence staining pattern of glomeruli from tumor-progressor mice is shown in Fig. 2. The deposits were clearly granular in nature and appeared in 100% of the glomeruli in affected mice. Since an underlying, age-associated immune-complex disease has been described in this mouse strain (15, 16) and was confirmed by these data, further studies were confined to mice younger than 16 wk of age.

It was necessary to determine whether the increased intensity and GBM involvement of immune-complex deposition in tumor-progressor mice represented acceleration of an ongoing disease due to the physiologic stress of a growing

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2 Poskitt, P. K. F., T. R. Poskitt, and J. H. Wallace. 1974. Spontaneous release of membrane-associated tumor-specific antigen by cultured B-16 melanoma cells. Manuscript submitted for preparation.
TABLE I
Age- and Tumor-Related Renal Immunoglobulin Deposition

| Tumor status                                | Age | Intensity and distribution* of renal immunoglobulins |
|---------------------------------------------|-----|-----------------------------------------------------|
|                                             |     | IgG  IgM  IgA                                      |
| Control (not challenged)                    |     |                                                   |
| 11                                          |     |                                                   |
| wk                                          |     |                                                   |
| 11                                          | 0   | 0         | 0         |
| 11                                          | 0   | 0         | 0         |
| 11                                          | 0   | 0         | 0         |
| 11                                          | 0   | 0         | 0         |
|                                             | 22  | 1+ m     | tr m     | tr-1+ m  |
|                                             | 22  | 1+ m     | 1+ m     | 1+ m     |
|                                             | 22  | 1+ m     | 1+ m     | tr m     |
|                                             | 22  | 1+ m     | 1+ m     | tr m     |
| Bearing a progressive subcutaneous tumor    |     |                                                   |
| 11                                          | 3+ g| 1-2+ m, g| tr m     |
| 11                                          | 2-3+ m, g| 2-3+ m, g| tr m     |
| 11                                          | 2+ m, g  | 2+ m, g  | tr-1+ m  |
| 11                                          | 2+ m, g  | 1+ m, g  | tr m     |
| 11                                          | 2+ m, g  | 2-3+ m, g| tr m     |
|                                             | 22  | 3+ m, g  | 2+ m, g  | 2-3+ m, g|
|                                             | 22  | 2-3+ m, g| 1+ m     | 1+ m, g  |
|                                             | 22  | 2+ m, g  | tr-1+ m  | 2+ m, g  |
|                                             | 22  | 2+ m, g  | tr-1+ m  | tr-1+ m  |

The values are shown for individual animals. Tumor-bearing animal’s kidneys were examined 20-25 days after s.c. challenge with 1 × 10⁶ B-16 cells.

* m indicates mesangial localization of deposits, g indicates GBM localization of deposits.

The paper discusses the presence of immune complexes in malignant melanoma, focusing on the relationship between renal immunoglobulin deposition and tumor status. The data in Table I show that renal IgG deposits are more intense in animals with progressive subcutaneous tumors compared to control animals. Animals sensitized and challenged with soluble tumor antigen all showed greater than 2+ IgG immune complex deposition as did animals bearing progressive tumor. However, animals which spontaneously rejected the tumor and tumor-progressor animals which had been pretreated with a dose of irradiation which preferentially suppresses primary immune responses all showed renal IgG deposits of 1+ or less. These results suggested that the renal deposits might represent soluble circulating complexes of tumor antigen and antitumor antibody.

Immunoglobulin was eluted from pooled kidney tissue of 9-wk old tumor-bearing animal's kidneys were examined 20-25 days after s.c. challenge with 1 × 10⁶ B-16 cells.
Fig. 2. (a) Tumor-progressor glomerulus stained with anti-IgG FITC showing granular mesangial and GBM deposits. (b) Tumor-progressor glomerulus stained with anti-IgM FITC. Deposits are primarily mesangial. (c) Tumor-progressor glomerulus stained with anti-IgA FITC. Deposits are primarily mesangial. (d) Age-matched nontumor-bearing control stained with anti-IgM FITC. × 200.
Table II

Renal IgG Deposition in 11-Wk Old Tumor Challenged and Soluble Antigen-Sensitized Mice

| Group                                      | Fraction with renal IgG* deposits scored as: | Group average ab. titer† |
|--------------------------------------------|---------------------------------------------|--------------------------|
| Tumor progressors                          | ≤1+ 0/5, >1+ ≤2+ 0/5, >2+ 5/5               | 1:200                    |
| Soluble antigen-sensitized §                |                                            |                          |
| Tumor progressors (immunosuppressed[])      |                                            |                          |
| Tumor rejectors                            | 5/5, 0/5, 0/5                              | <1:10                    |
| Control                                    |                                            |                          |

* Renal IgG deposits determined by indirect immunofluorescence.
† Serum antibody (ab.) titer determined by mixed hemadsorption assay.
§ 50 μg of soluble B-16 antigen given s.c. in adjuvant day 0; 150 μg given i.p. day 22.
|| 450 R whole-body irradiation 2 days before challenge. Serum and kidneys were examined 24 days after the initial sensitization or challenge. The classification of progressors and rejectors is based on the presence or absence of a palpable tumor at the time of examination. In our experience, animals which have palpable tumors at this stage always die of the tumor. Animals which do not have palpable tumors by day 24 always survive the tumor and are resistant to subsequent challenges.

Fig. 3. Indirect immunofluorescence for IgG antibody activity against B-16 cell membranes. (a) Pooled (KI and citrate) renal eluate from tumor-progressor mice. (b) Pooled undiluted serum from tumor-progressor mice. (See Table III for titers and specific activity).

Progressor mice and examined by indirect immunofluorescence for reactivity with the B-16 tumor cell membranes. Tumor-bearing mice in this age group showed only IgG in renal deposits. The fluorescent staining pattern is shown in Fig. 3. Table III shows the relative activity and specificity for B-16 cells of renal eluates and serum derived at the same time from the same animals. The specific activity for B-16 cell membranes of IgG in the pooled KI and citrate eluates was 32 times greater than in the serum (relative to the IgG concentration). Neither the serum nor the pooled eluate reacted with C57 liver cells or
erythrocytes in an indirect immunofluorescence assay and the activity against B-16 cells could not be removed by absorption with C57 tissue powder. IgM and IgA could not be detected in the eluates and no IgM or IgA antibody was detected in the indirect fluorescence assay with B-16 cells, C57 liver cells, or erythrocytes.

The relationship of the renal IgG deposits to tumor size, intensity of mononuclear leukocyte (MNL) infiltration, and the presence of lung metastases is shown in Table IV. In general, mice which showed only small amounts (≤ 1+)

| TABLE III | Antibody Activity of Plasma and Renal Eluates from Tumor-Bearing Mice |
|-----------|-------------------------------------------------------------------|
| Sample                | Absorption*            | IgG titer vs. B-16 | Specific activity§ |
| Plasma               | —                     | 8                  | 1                   |
| Citrate eluate       | —                     | 2                  | 32                  |
| KI eluate            | —                     | 4                  | 64                  |
| Plasma               | C57 tissue powder     | 8                  | 1                   |
| Pooled KI and citrate eluate | C57 tissue powder | 2                  | 32                  |
| Plasma               | B-16 cell powder      | 0                  | 0                   |
| Pooled KI and citrate eluate | B-16 cell powder | 0                  | 0                   |

* Absorption carried out with equal volumes of plasma or eluate and lyophilized tissue powder.

† IgG titer vs B-16 determined by indirect immunofluorescence. Similar tests using anti-IgM or IgA were negative. Indirect fluorescence was negative for all three immunoglobulin classes against C57 liver cells and erythrocytes.

§ IgG anti-B-16 specific activity relative to unabsorbed plasma.

Spec act = \( \frac{\text{eluate titer}}{\text{plasma IgG concentration}} \times \frac{\text{plasma titer}}{\text{eluate IgG concentration}} \)

| TABLE IV | Relationship of Renal IgG Deposits to In Vivo Parameters of Tumor Growth |
|-----------|------------------------------------------------------------------------|
| Renal IgG deposits scored as: | ≤1+ | >1+ ≤2+ | >2+ |
| Fraction of tumor progressors in each category | 13/33 | 10/33 | 10/33 |
| Fraction of animals with MNL infiltrate of tumor scored as: | | | |
| None | 0/13 | 1/10 | 5/10 |
| Mild | 1/13 | 4/10 | 5/10 |
| Moderate | 7/13 | 4/10 | 0/10 |
| Pronounced | 5/13 | 1/10 | 0/10 |
| Fraction of animals with lung metastases | 0/13 | 1/10 | 5/10 |
| Tumor volume (mm³) Range | 150-1,800 | 455-1,800 | 560-5,100 |
| Average | 722 | 998 | 2,272 |

Eight age-matched control mice showed renal IgG of 0-tr.
of renal deposits of IgG had relatively small tumors at the injection site and the
tumors had moderate to pronounced MNL infiltration (Fig. 4 a). A correlation
between a favorable prognosis and intense lymphocytic infiltration has been
reported in human malignant melanoma (17). None of these animals had meta-
tases to the lungs. Mice which showed moderate (>1+ ≤2+) renal IgG de-
posits had somewhat larger tumors with relatively less MNL infiltration. One
animal had lung metastases. Mice which had large (>2+) amounts of renal
IgG had the largest tumors with little or no MNL infiltration (Fig. 4 b) and 5
out of 10 had microscopic lung metastases. All parameters were examined at
24–26 days after tumor inoculation.

The extreme variability (none to pronounced) of the intensity of MNL in-
filtration of the tumors suggests that there is a difference in the cell-mediated
tumor rejection response in these animals which is related to immune complex
deposition. However, in vitro testing of PBL from similar animals (Table V)
revealed that all tumor-progressor animals had some degree of PBL cyto-
toxicity against B-16 cells at day 20 after tumor inoculation. PBL cytotoxicity
was not found in any animals at day 10. The intensity of day 20 PBL reactivity
did not correlate with the tumor size at day 20 or with the time of death.
Furthermore, animals which spontaneously rejected the tumor had essentially
identical PBL reactivity as did animals which succumbed to the tumor.

Humoral antibody activity in the tumor-progressors and tumor-rejectors is
shown in Table VI. Day 20 post-tumor inoculation antibody titers of individual
tumor-progressor plasmas did not differ significantly. Both cytotoxicity and
blocking activity could be demonstrated in the same plasma at the same dilution
in some of the progressor animals. In some cases, where blocking activity was
not found, the plasma and PBL combination resulted in enhanced (greater than
additive) cytotoxicity implying perhaps an antibody-dependent cellular cyto-
toxicity. Animals which had cytotoxic antibody or whose plasma enhanced
cellular cytotoxicity did not survive significantly longer than those which did not;
nor did the presence of plasma blocking activity correlate with shortened
survival time. Tumor-rejectors, as a group, had no detectable antitumor anti-
body at day 20 by mixed hemadsorption, cytotoxic, or blocking assays. Mice
sensitized to the B-16 tumor with either killed (irradiated or frozen) tumor cells
or with soluble tumor antigen developed both cellular and humoral responses
comparable to those in tumor-progressor animals. When the sensitized animals
were subsequently challenged with a tumor inoculum (5 x 10^4 cells) which did
not take in control animals, they all developed progressive, lethal tumors
(Table VII). These animals showed the most pronounced (all >2+) renal IgG
complex deposition and the least amount of MNL infiltration of the tumors
of any animals tested.

Of the five unfractionated plasma pools tested, tumor-specific antigen was
found only in one. This was the pool consisting of day 10 serum from tumor-
progressor mice which had not shown antibody activity. However, after frac-
Fig. 4. Hematoxylin and eosin-stained sections of subcutaneous B-16 melanoma. (a) from a representative animal with >2+ renal IgG showing healthy tumor cells around blood vessel with no MNL infiltrate. × 200. (b) From a representative animal with <1+ renal IgG showing MNL infiltrate surrounding blood vessel and dead or dying tumor cells in the vicinity of the infiltrate. × 200.
**IMMUNE COMPLEXES IN MALIGNANT MELANOMA**

**TABLE V**

*Relationship of In Vitro PBL Cytotoxicity to Tumor Progress*

| Tumor status | PBL % cytotoxicity* | Day of death |
|--------------|---------------------|--------------|
| Progressor   |                     |              |
|              | 76                  | 33           |
|              | 54                  | 25           |
|              | 54                  | 30           |
|              | 53                  | 38           |
|              | 38                  | 50           |
|              | 37                  | 42           |
|              | 31                  | 37           |
| Rejector     | 49                  | -            |
|              | 47                  | -            |
| Control      | 8                   | -            |

* In vitro PBL cytotoxicity vs. [3H]TdR-labeled B-16 cells. All animals were tested individually on day 20 post-challenge. Control are animals which were not tumor challenged.

**TABLE VI**

*Relationship of Humoral Antibody to Tumor Progress*

| Tumor status | Ab. titer* | Cytotoxicity† | Blocking activity§ | Day of death |
|--------------|------------|---------------|-------------------|--------------|
| Progressor   | 320        | ++ + +        | + + +             | 42           |
|              | 320        | 22            | 0                 | 33           |
|              | 160        | 22            | + + +             | 38           |
|              | 160        | 25            | + +               | 25           |
|              | 160        | 28            | + + + +           | 30           |
|              | 160        | 0             | 0                 | 50           |
|              | 80         | 0             | 0                 | 37           |
|              | <10        | 0             | 0                 | -            |
| Rejector     | <10        | 0             | 0                 | -            |
| Control      | <10        | 0             | 0                 | -            |

All animals were tested individually on day 20 post-challenge. Controls are animals which were not tumor challenged.

* Reciprocal of last positive dilution in mixed hemadsorption assay vs. B-16 cells.
† Percent [3H]TdR-labeled B-16 cells killed by a 1:10 dilution.
§ Percent inhibition of autologous PBL cytotoxicity by a 1:10 dilution. 0, none; +, ≤25%; ++, >25% ≤50%; ++++, >50% ≤75%; +++++, >75%.
TABLE VII

Effect of Presensitization on Take and Progress of Subsequent Tumor Challenge

| Treatment group                        | No. animals | No. takes | Time of take* | Time of death* | No. survivors |
|----------------------------------------|-------------|-----------|---------------|----------------|---------------|
| Saline control                         | 8           | 0         | —             | —              | 8             |
| Multiple injections of killed tumor    | 10          | 10        | 15 (10-17)    | 29 (25-34)     | 0             |
| Multiple injections of soluble tumor    | 6           | 6         | 13 (10-16)    | 28 (26-30)     | 0             |

Animals challenged with $5 \times 10^4$ live B-16 (s.c.) 2 wk after the last immunizing injection.

* Indicates days after tumor challenge. The average and range are shown for the day of take (palpable tumor) and death.

Discussion

We have reported previously that in vitro cultured B-16 cells spontaneously release a soluble, tumor-specific membrane antigen (20,000-27,000 mol wt), and that antigen release occurs both through active metabolism and autolysis. It appears that a similar antigen (less than 50,000 mol wt) is shed in vivo, as evidenced by the detection of free or antibody-complexed antigen in the serum and in renal deposits of tumor-progressor animals. Furthermore, the intensity of renal immune complex deposition showed a more consistent correlation with in vivo parameters of tumor growth (size and metastatic spread) and host rejection response (MNL infiltration) than did in vitro assays for peripheral blood lymphocyte cytotoxicity, antibody titer, cytotoxic antibody or blocking antibody. Lymphocyte cytotoxicity assays at a low lymphocyte: target cell ratio (2:1) failed to discriminate between tumor-progressors and rejectors, and the level of cytotoxicity did not correlate with survival time in progressors. Tumor-reactive antibody (mixed hemadsorption assay) was consistently found in progressor animals; however, functional assays for cytotoxicity and blocking activity were poor indicators of tumor progress with regard to the most significant criterion, namely, survival.

The existence of tumor antigen in the serum of tumor-bearing individuals has been described previously (11, 12, 18, 19) and such serum has been shown to block in vitro lymphocyte cytotoxicity presumably by interaction with the lymphocyte antigen receptors (11, 12, 18). In other systems, however, humoral blocking activity appears to be a property of noncytotoxic antibody which masks the tumor cell antigens (20). The definition of "blocking" activity is quite dependent on the way the serum is assayed. Thus, if serum is preincubated with target cells and removed before addition of the lymphocytes, only "masking
antibody” can be detected. If the serum is preincubated with the lymphocytes, on the other hand, only antigen blockade of T-lymphocyte receptors can be detected.

Fig. 5 shows the interactions which might occur between soluble circulating tumor antigen and immune effectors. In this scheme, the biological effects of antibody and T lymphocytes are proposed to be controlled by fluctuating levels of soluble antigen. In conditions of antigen excess, free or antibody-complexed antigen may bind to T-lymphocyte antigen receptors and thus activate the cells.

These lymphocytes would be stimulated at a distance from the tumor mass and would therefore expend their short-range effectors (MIF, lymphotoxin, etc.) without causing damage to the tumor. Thus, one might expect that in individuals with massive tumor burdens delayed hypersensitivity responses to additional tumor antigen might be minimal or absent. In a study of eight patients with malignant melanoma, Fass et al. (21) found that only those with small, localized tumors (3/8) showed delayed hypersensitivity reactions to autologous tumor cells. The five patients with disseminated disease were unreactive. These results were not a reflection of general anergy as all eight patients showed normal delayed hypersensitivity reactions to other antigens to which they were previously sensitive.

Furthermore, antigen-antibody complexes bound by T lymphocytes might result in complement fixation and lysis of the cells. This could account for the
lesser reactivity in vitro of washed lymphocytes from melanoma patients with disseminated tumors (22). Thus, it is possible to deplete the effective T-lymphocyte population to the point where even a strongly sensitized individual will appear unreactive.

In the B-16 system, the C57BL/6 host does not have a complete hemolytic complement system and so depletion of reactive lymphocytes by lysis may not occur. In fact, when peripheral blood lymphocytes were removed from animals with varying degrees of tumor mass and washed thoroughly, they behaved very similarly. On the other hand, the in vivo effectiveness of the lymphocytes as measured by MNL infiltration of the tumor was markedly different in animals with large and small tumors. Thus, the potential activity of the lymphocytes was the same regardless of tumor mass, but their actual effectiveness was diminished in animals with larger tumors. This suggests the possibility that diminished lymphocyte reactivity in vivo could be an effect rather than a cause of rapid tumor growth and consequent release of large amounts of tumor antigen.

Referring again to Fig. 5, in conditions of slight antibody excess, the phenomenon of masking tumor cell antigens by antibody present in insufficient quantities to be cytotoxic (by complement fixation or antibody-dependent cellular cytotoxicity) may occur. Whereas, in conditions of extreme antibody excess, brought about by removal of part of the tumor mass and decrease in available circulating antigen, the same antibody which previously "masked" might become cytotoxic. For example, Lewis et al. (23) have reported the appearance of cytotoxic antibody in two previously negative patients following partial excision of their malignant melanoma. Furthermore, inhibition of serum-blocking activity by the serum of patients in remission (24) may reflect the attainment of a balance of antigen and antibody in the mixture of the two types of sera resulting in effective neutralization of both antigen and antibody.

The postulated interaction presented in Fig. 5 is not meant to represent alternative static conditions, but rather a cycle which is controlled chiefly by the availability of circulating antigen. Since we have shown\(^2\) that soluble antigen can be released by autolysis following tumor cell death as well as by living cells, it is possible that vigorous immunologic attack of the tumor may be ultimately self-defeating by accelerating the release of soluble antigen.

Monitoring of renal immune complex deposition in one reported case of Hodgkin's disease (5) proved to be useful for following and, in fact, predicting the course of the disease. At present, renal biopsies are usually performed only on cancer patients with obvious renal complications; however, the study reported here suggests that significant immune complex deposition can occur in tumor-bearing individuals without clinical evidence of renal disease. Examination of this phenomenon in a selected series of cancer patients could establish its general occurrence and possible prognostic value.
IMMUNE COMPLEXES IN MALIGNANT MELANOMA

SUMMARY

Histologic and immunofluorescence studies of the kidneys of mice bearing a progressive melanoma show a proliferative glomerulonephritis associated with immune complex deposition in the mesangium and along the glomerular basement membrane. This immune complex disease is distinct from the age-associated disease of the C57BL/6J host strain and the complexes can be shown to consist of soluble tumor antigen and antitumor antibody. Furthermore, the intensity of IgG complex deposition correlates directly with tumor progress (size and metastases) and inversely with mononuclear leukocyte infiltration of the tumor. In vitro assays for lymphocyte cytotoxicity and humoral antibody were found to be less reliable indicators of tumor progress. The possible role of circulating soluble tumor antigen in modifying the immune response to tumors is discussed.

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