Histocompatibility Typing and Course of Canine Venereal Tumors Transplanted into Unmodified Random Dogs

Robert B. Epstein and B. Taylor Bennett

Veterans Administration West Side Hospital, and Departments of Medicine [R. B. E.] and Pathology [B. T. B.], Abraham Lincoln School of Medicine, University of Illinois, Chicago, Illinois 60612

SUMMARY

The clinical course following transplantation and the histocompatibility specificities were compared for two cell lines of the canine transmissible venereal tumor. The naturally occurring tumors originated in dogs from Malaya and Chicago. All of the 71 dogs challenged with either the Mafkyan or Chicago cell line developed tumors. The clinical courses of both tumor lines were similar and included periods of logarithmic growth, stability, and regression. Four dogs showed accelerated growth or metastatic disease. Histocompatibility antigenic specificity was tested on tumor cells by the cytotoxicity reaction with the use of a panel of 64 canine allotypic antisera. Specificities for histocompatibility Groups 3, 10, and 8 were interpreted to be present on both cell lines. Cells reacted identically with 62 of the 64 antisera. Four sera obtained from animals following tumor regression showed associated allotypic specificities when tested against the lymphocytes of normal dogs. Elution and immunofluorescent studies demonstrated membrane-associated IgG on tumor cells 7 days following transplantation which increased with tumor age and decreased with dissemination. It was concluded that cells obtained from tumor sources of widely disparate geographical origin present similar clinical and immunogenic characteristics. The tumor system may be useful for studies of immunological mechanisms involved in tumor growth in unmodified recipients of a randomly bred species.

INTRODUCTION

Overt transmission of neoplastic tissue in randomly bred mammalian species is rare (14). One notable exception is the canine TVT4 first described by Novinsky in 1876 (10). Clinical and immunological characteristics of the tumor were described as early as 1906 by Sticker (15) and, more recently, by DeMonbreun and Goodpasture (4), Powers (11), and Cohen (2). The tumor is an undifferentiated, round-cell neoplasm which normally affects the external genitalia of both sexes and is transmitted by sexual contact in nature (13). Regression after a variable period of time is common, but fatal metastatic disease may occur (12). The tumor has worldwide distribution (9).

The explanation for the unique natural history of this neoplasm remains obscure. Cohen (2), using immunofluorescent techniques, demonstrated the appearance of membrane-specific IgG on tumor cells approximately 40 days following transplantation. Sera from regressor animals were capable of producing positive indirect immunofluorescence on cells less than 30 days posttransplantation (2). The relation of the antibody response to normal canine histocompatibility antigens or to possible tumor-specific antigens is unclear.

The recent identification of a DL-A system in dogs (18, 19) suggests a basis for determination of canine histocompatibility specificities on TVT cells. Such specificities, in addition to providing a marker system for cells from various tumor lines, may yield information regarding mechanisms of growth of this neoplasm transmitted between members of a genetically heterogeneous population.

These studies were carried out to establish basic information on the natural history following experimental transplantation of TVT cells in unmodified random dogs. Cell lines obtained from naturally occurring tumors in a dog from Malaya and a 2nd dog from the Chicago area were studied. Testing with a panel of canine histocompatibility typing antisera was performed to identify the presence of DL-A antigens on cells the 2 tumor lines. In addition, tumors were examined by elution and immunofluorescent techniques for the presence of membrane-associated IgG during the course of neoplastic growth and regression.

MATERIALS AND METHODS

Random mongrel dogs weighing between 6 and 15 kg, 4 months to 1 year of age, were used in the studies. Dogs were routinely dewormed, immunized against hepatitis and distemper, isolated from new dogs entering the colony, and observed for a period of 3 to 4 weeks. Dogs of both sexes were used. Females were judged to be nulliparous on the basis of being raised in the kennel or by physical examination.
Tumor Cell Preparation. Two tumor lines were studied. The 1st cell line originated in a dog with naturally occurring TVT from Malaya (MTVT). This tumor had undergone at least 20 passages before cells were isolated, cryopreserved, and shipped to this laboratory. Initial transplantation was carried out with $10^8$ rapidly thawed tumor cells injected into 2 s.c. sites of a random mongrel recipient. The 2nd tumor line (CTVT) originated in a clinical case occurring in an 18-month-old nulliparous female mongrel referred to this facility in Chicago in November 1971. Initial transmission was carried out with trypsinized cells obtained from biopsied tissue. Subsequent to tumor growth of these 2 tumor lines in the primary recipients, further transmission was standardized in the following way. Surgical biopsies were placed in buffered Hanks' solution containing 0.25% trypsin and a few drops of a 0.02% DNase solution (2).

Trypsinization of the mass was carried out at room temperature with a magnetic stirrer. At 20-min intervals, the cell-rich supernatant was filtered through sterile gauze mesh. The 1st material obtained was discarded and the process was repeated until sufficient numbers of cells were obtained for transplantation and serological studies. The tumor cells were washed twice in Hanks' solution and concentrated by centrifugation to $10^6$ cells/ml. Recipient dogs received a dose of $10^8$ tumor cells at 4 separate s.c. sites. Some cells from each biopsy were utilized for histocompatibility testing.

Histocompatibility Testing. A panel of 64 canine cytotoxic antisera were tested against the 2 tumor lines on a total of 75 separate biopsy specimens obtained from 40 random dogs. A modification of the 2-stage microcytotoxicity technique described by Terasaki and McClelland (16) was used. Canine lymphocyte cytotoxicity testing was performed as previously described (18). Antisera used in the panel have been shown to recognize DL-A specificities by selection of histocompatible littermates for tissue grafting and by genetic studies of families and random dogs (5, 19, 20).

Sera obtained from 4 animals following tumor regression were also tested against random dog lymphocytes. Absorption of antisera was performed as described by van Rood (17).

Clinical Evaluation. Following tumor inoculation, dogs were inspected daily until a measurable tumor was present, and were inspected weekly thereafter. Measurement of the 4 representative tumor masses was carried out by caliper in 3 perpendicular directions, and the tumor volume in cu mm was calculated (3) as

$$V = \frac{\pi}{6} \times \left( \frac{D_1 + D_2 + D_3 - D_4}{3} \right)^3$$

Elution Studies. For elution studies, tumor tissue was minced in phosphate-buffered saline at pH 7.4 and then homogenized in a Teflon Procter-Elvehjem tissue grinder with 1 passage of the grinder over the tissue pieces. The cells were filtered through a thin layer of glass wool and washed 6 times with phosphate-buffered saline. To 1 part packed cells were added 2 parts glycine: HCl-buffered saline at pH 2.9, and the mixture was incubated for 1 hr at 37°. After incubation, the mixture was centrifuged, the supernatant was put aside, and the process was repeated. The supernatant fluids from both incubations were neutralized to pH 7.0 with 0.1 M NaOH and concentrated by vacuum dialysis against phosphate-buffered saline, pH 7.4. The concentrate was subjected to immunoelectrohoresis, with 0.03 M barbital buffer at pH 8.2, and developed with rabbit anti-canine IgG and anti-canine serum.

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RESULTS

Clinical Course of the Tumor Transplantation in Unmodified Recipients. All animals challenged with the standard inoculum of TVT cells developed a clinically palpable tumor within 10 days. This occurred in a total of 71 dogs challenged at over 300 s.c. sites. Following the appearance of a palpable mass, a period of logarithmic growth occurred, followed by a period of relative tumor stability and subsequent regression. Table 1 summarizes the duration

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of phases for CTVT and MTVT dogs. Ranges for growth, stability, and regression varied considerable within both groups but were comparable for the 2 tumor lines. Ten dogs with MTVT and 6 dogs with CTVT were sacrificed after 150 days during the period of stability. Chart 1 compares the course of measured tumor growth, followed to complete regression, in 6 dogs with MTVT and 6 dogs with CTVT. Local tumor masses reached sizes between $10^9$ and $10^6$ cu mm in both groups. With each tumor line, relatively early and late regressors were seen.

The effect of surgical biopsy on tumor growth was assessed in 16 biopsied and 12 nonbiopsied dogs. In all instances surgery was performed between 20 and 35 days when tumors had reached sizes of over $10^3$ cu mm. No significant influence of the surgical procedure was apparent on subsequent tumor growth followed for at least 150 days.

Following regression, 8 dogs were rechallenged with a standard tumor inoculum. In 2 instances, dogs rejecting CTVT received MTVT cells, and vice versa. All dogs so challenged demonstrated no evidence of tumor growth. Multiple biopsies were obtained on dogs during the course of tumor growth and rejection. Fig. 1, A and B, illustrated the histological similarities of the 2 tumor lines during the period of rapid growth. Fig. 1 C shows the invasion by a lymphocytic, plasma cellular infiltrate occurring during early regression, and Fig. 1 D shows the histological picture of the end stage of the process.

Tumor Regrowth and Metastasis. Four dogs varied from the usual pattern of tumor growth and regression. In 2 of these, a period of tumor regression and stability was followed by a 2nd phase of rapid progression. Ultimately, these animals were sacrificed at Day 300 with extensive local invasive disease.

In 2 animals, metastatic disease was encountered. At 232 days posttransplantation, Dog 1048 developed a metastatic lesion that involved the left tonsillar crypt and the cervical lymph nodes. The metastatic growth reached a maximum size of $10^6$ cu mm and after 51 days began to regress. By Day 309, the animal was tumor free.

In Dog 1513, metastatic disease developed in the pre-scapular and inguinal nodes 8 months following transplantation. The lesions continued to grow, and the dog was sacrificed on Day 330 in a moribund state. At necropsy, a metastatic tumor was found in the right apical lobe of the lung.

Tumor-typing Results. Table 2 summarizes the results of tumor testing with a panel of 64 canine lymphocyte cytotoxic antisera utilized in histocompatibility studies of families and random dogs for transplantation purposes. Fifty antisera could be associated with previously described DL groups of either the 1st or 2nd series (19). The remaining 14 antisera have broad specificities or have not yet been assigned to definite groups. Clearest readings were obtained on tumors 4 to 6 weeks posttransplantation. Older tumors frequently gave positive control or ambiguous results. In general, the strength of the serological reactions was less than that noted in lymphocyte testing, causing difficulties in interpreting reactions with some antisera. With repeated testing, however, both tumor lines were positive with antisera of DL-3 and -10 and were weakly reactive with 2 of 3 DL-8 antisera. Positive reactions were noted with unclassified antisera usually of broad specificity (Table 2). Consistent differences in reactions between the cells of the tumor lines were observed with 2 of the 64 antisera tested. The overall DL-A results were interpreted to indicate a high degree of similarity between MTVT and CTVT for specificities recognized by the typing panel.

Following tumor regression, sera from 4 animals were tested for allospecificity against normal lymphocytes. Initially, 20 random dogs were screened in the cytotoxicity test. Allotypic specificities could be identified on normal canine lymphocytes with the antisera. A total of 105 random dogs were then typed with the 4 regressor antisera, and the resulting reactions were analyzed by 2 x 2 contingency tables, as described by van Rood (17). Table 3 shows the $x^2$ values for independence between serum pairs, suggesting that they recognize associated DL-A specificities.
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Table 2
Cytotoxicity testing of CTVT and MTVT tumor cells

<table>
<thead>
<tr>
<th>DL group</th>
<th>No. of sera tested</th>
<th>CTVT</th>
<th>MTVT</th>
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<tbody>
<tr>
<td>1st series</td>
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<td>2</td>
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<tr>
<td></td>
<td>2</td>
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<td>+</td>
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<td>9</td>
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<tr>
<td></td>
<td>C16</td>
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</table>

* ±, doubtful reaction.
* Weak reactions with 2 of 3 antisera.

\[ \chi^2 \] associations between antitumor antisera

Antisera obtained from dogs following tumor regression were tested against lymphocytes from 105 normal dogs.

<table>
<thead>
<tr>
<th>Antisera</th>
<th>819</th>
<th>1680</th>
<th>1885</th>
<th>3.9</th>
<th>3.3</th>
<th>4.0</th>
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<tbody>
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<td>819</td>
<td>1680</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

* OD, antisera obtained from the dog with the naturally occurring Chicago tumor.

In randomly bred mammalian species, the highly polymorphic nature of the histocompatibility loci provides for the almost universal rejection of allogenic grafts by unmodified recipients. It has been postulated that the polymorphism of the histocompatibility system serves a useful purpose in nature by preventing ready cell transmission of malignant neoplasms (14). One of the exceptions to this general rule is found in the canine TVT as occurs in nature and in laboratory studies. Cell-free extracts have failed to produce tumor, and recent chromosomal studies strongly support a cellular mode of transmission (1, 6). In the present study, transmission was accomplished in all instances by tumor cell transfer. The regularity of such transmission could imply either a lack or modification of histocompatibility antigens on the tumor surface, or transmission of an infectious agent. These studies clearly indicate that the tumor cells derived from 2 widely disparate canine sources possess a complement of antigenic specificities that can be identified in cytotoxicity testing by DL-A typing antisera. With a panel of such antisera, almost identical typing patterns were obtained with both tumor lines. These specificities remained constant throughout 40 cell passages. Since the naturally occurring tumor is most likely a cell transplantation itself, the present laboratory manipulations and cell passages are basically analogous to the wild setting. Cytotoxic testing thus provides a marker system for these cells and may shed some light suggesting a common origin of this widely distributed canine tumor.

The present studies show that ultimate rejection of the tumor in the canine probably involves the DL-A system. Rejecting animals develop allotypic lymphocyte typing antisera. Antisera so developed showed significant degrees of \[ \chi^2 \] associations in 2 \( \times \) 2 contingency tables (17). Studies are underway at this time to explore the relationship of these DL-A antisera to currently established DL groups, to study their usefulness in histocompatibility segregation studies in families, and to correlate the natural history of the tumor of DL-A typing.

Although mobilization of an immune response against tumor cells based on histocompatibility antigens probably occurs, modification of the allograft response must be present to account for the variability described in the
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clinical course of the tumor. The relation of DL-A and/or tumor-specific antibodies to the course of the tumor remains to be clarified.

These studies confirm and extend the work of Cohen (2) describing the evolution of an IgG antibody in the sera of dogs after approximately 40 days of tumor growth. The current studies indicate that, very early in tumor growth (7 days), canine IgG can be eluted from the tumor masses. In addition, direct immunofluorescence confirmed the presence of an IgG membrane-associated antibody during the period of rapid growth.

Although precise quantitative studies were not done, immunoelectrophoresis of tumor eluates and observations of immunofluorescence showed an increasing amount of IgG throughout the period of growth. Of interest was Dog 1513 that, following the onset of regression, later developed rapid tumor progression and fatal metastatic disease. Serial determination of fluorescence indices in this dog showed an almost complete disappearance of the circulating antibody prior to clinically detectable tumor progression. Similar falls in tumor-associated antibody levels occurring with dissemination have also been reported in human sarcomas (8).

The canine venereal tumor may be useful for future studies of the relationship of transplantation antigens to mechanisms of tumor growth in a randomly bred species.

ACKNOWLEDGMENTS

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REFERENCES

Fig. 1. Histological evolution of the transplanted tumor: 
A, CTVT in growth phase; B, MTVT in growth phase; C, tumor undergoing early regression with lymphocytic and plasma cellular infiltration (Day 126); and D, tumor site following clinical regression. H & E, × 320.

Fig. 2. Immunoelectrophoresis of tumor eluate. Precipitin reactions are shown with anti-canine IgG and anti-canine whole serum (cs).
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