Evaluation of Growth and Histology of Human Tumor Xenografts Implanted under the Renal Capsule of Immunocompetent and Immunodeficient Mice

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ABSTRACT

Fresh surgical explants of human carcinomas were implanted as first transplant generation xenografts under the kidney capsule of mice. Immunocompetent and immune-deficient mice were compared for their ability to support the persistence and growth of these xenografts. Consistent growth of tumor xenografts could not be demonstrated following implantation under the kidney capsule of immunocompetent mice. Immunological infiltration and rejection of the xenografts began 3 days postimplantation, and tumors were largely eliminated from the subcapsular space by 6 days postimplantation. In contrast human tumors consistently grew under the kidney capsule of nude mice. Significant growth became apparent by 9 days postimplantation with most human carcinomas and continued thereafter. Growth was always accompanied by neovascularization of tumor xenografts which was visible by examination of tumor-bearing kidneys under a dissecting microscope (× 6). There was no histological evidence of immunological interference with the persistence and growth of xenografts in nude mice. Thymectomized, irradiated, bone marrow-reconstituted conventional mice, as well as conventional mice, treated daily with 60 mg of cyclosporine A/kg were comparable to nude mice as hosts which supported the long-term persistence and growth of subrenal capsule implants of human tumors. Such mice could provide an alternative to nude mice as hosts in which chemosensitivity assays could be carried out against growing human tumors at a considerable saving in cost and convenience.

INTRODUCTION

The SRC3 is being used as an in vivo test of human tumor responsiveness to drug therapy (1–7). Pieces (1 mm³) of solid tumor are implanted as xenografts under the kidney capsule of mice. Tumor-bearing mice are treated with a particular cancer chemotherapeutic agent, and change in tumor size is recorded as a reflection of tumor sensitivity to that agent. Bogden et al. (1) developed the SRC in congenially athymic (nude) mice because of their inability to mount a host versus graft reaction against the human tumor xenograft. These investigators have since adapted this assay to normal, immunocompetent mice because of the high cost of nude mice (8). They reported that the host versus graft response in the subrenal capsule environment of immunocompetent mice did not significantly inhibit xenograft persistence during the first week after grafting. This conclusion was based on the observation of similar and in some cases greater size increases in human tumor xenografts in normal, immunocompetent mice compared to that found in nude mice in a 6-day assay (8). Several investigators have begun using this assay to test the chemosensitivity of human and experimental tumors xenografted into the subrenal capsule space of either nude mice (2) or immunocompetent mice (3–7, 9, 10).

Recent histological studies by Edelstein et al. (3), Bennett et al. (11), Dumont et al. (6), and Levi et al. (5) have demonstrated significant host cell infiltration of human tumor xenografts 6 days after implantation under the renal capsule of immunocompetent mice, and have suggested that this infiltration could contribute to size changes in the xenografts which could complicate interpretation of chemosensitivity data obtained in the assay. The present study was designed to compare immunocompetent mice to nude mice as hosts for human tumor xenografts implanted under the kidney capsule. The persistence and growth of these xenografts, as well as their induction of histopathological changes in the subrenal capsule environment during the first 3 weeks after implantation, are described. Regimens of immunosuppressing conventional mice which could make possible the substitution of immunosuppressed conventional mice for nude mice as hosts for human tumor xenografts are also described.

MATERIALS AND METHODS

Mice

Male C57BL × DBA/2 F₁ (hereafter called BD2F₁) mice were purchased from The Jackson Laboratory (Bar Harbor, ME) when they were 5 weeks old. Male Tac:N[NIH] Swiss nu/nu (nude) mice were purchased from Taconic Farms (Germantown, NY) when they were 5 weeks old. Nude mice were housed under positive laminar flow and handled using sterile technique. Mice were not used in experiments until they were 7 to 10 weeks old.

Immunosuppressive Regimens

Cyclosporine A. Cyclosporine A (Sandimmune i.v.) was purchased from Sandoz, Inc., East Hanover, NJ and was diluted in 0.9% NaCl solution. It was injected s.c. into BD2F₁ mice at a dose of 60 mg/kg every day for the duration of the experiment.

Thymectomy, Irradiation, and Bone Marrow Reconstitution. T-cell-deprived mice were created by thymectomy followed by lethal irradiation and bone marrow reconstitution. Normal 5-week-old BD2F₁ mice were thymectomized (12). Mortality rate from thymectomy was less than 10%. Fourteen days after thymectomy, mice were lethally irradiated (750 rads) and then reconstituted with 2 × 10⁶ syngeneic bone marrow cells given i.v. 24 h after irradiation. Bone marrow cells were pretreated with monoclonal anti-Thy-1.2 (Becton Dickinson) plus complement. TIB mice were used in experiments 1 to 3 weeks after bone marrow reconstitution.

Procurement of Human Tumor Specimens

Freshly resected human tumors were sectioned in the surgical pathology laboratory adjacent to the operating room suite under the guid-
ance of a surgical pathologist in order to select for tissue which contained a maximum of viable tumor cells and a minimum of necrotic tissue, stroma, and nonneoplastic cells. Frozen sections were examined microscopically to ensure an adequate yield of tumor cells in the piece selected for the SRC. Once the tumor piece had been selected, it was placed in a sterile specimen container, immersed in Hanks’ balanced salt solution containing penicillin (100 units/ml) and streptomycin (100 μg/ml), and brought to the laboratory for further dissection and implantation under the kidney capsule of mice.

Implantation of Human Tumors under the Kidney Capsule of Mice

The method of implanting human tumors under the kidney capsule of mice has been described by Bogden et al. (1, 8). Briefly, mice were anesthetized with Nembutal. The mouse kidney was exteriorized surgically, an incision was made in the capsule, and a 1-mm² tumor fragment was implanted under the capsule using a 16-gauge x 1.5-in. trocar. The size of the implant was determined in situ by measuring two perpendicular diameters to the nearest 0.1 mm using a dissecting microscope equipped with an ocular micrometer. The wound was closed with silk and wound clips, and each individual mouse was marked for identification. At various times after implantation, mice were sacrificed, the tumor-bearing kidney was exteriorized, a final measurement of xenograft size was taken, and the xenograft-bearing kidney was resected and placed in formalin. Change in xenograft size (ΔXS) was calculated using the formula:

\[ \Delta XS = \frac{L + W}{2} \text{ day } n - \frac{L + W}{2} \text{ day } 0 \]

where L and W represent two perpendicular diameters of the xenograft (8).

Histology

Preparation of slides for histology was carried out by cutting a 3-mm-thick cross-section containing the tissue tumor perpendicular to the long axis of each kidney. Each section was embedded in paraffin, serially sectioned by microtome, mounted on glass slides, and stained with hematoxylin and eosin. Histological studies provided qualitative results indicating that xenografts were occupied by varying amounts of tumor and stroma, and nonneoplastic cells. Frozen sections were examined microscopically to ensure an adequate yield of tumor cells in the piece selected for the SRC. Once the tumor piece had been selected, it was placed in a sterile specimen container, immersed in Hanks’ balanced salt solution containing penicillin (100 units/ml) and streptomycin (100 μg/ml), and brought to the laboratory for further dissection and implantation under the kidney capsule of mice.

RESULTS

Fresh surgical explants from two human adenocarcinomas of the colon (Chart 2A, A and B), and one small cell carcinoma of the lung (Chart 2C) were implanted as first transplant generation xenografts under the kidney capsule of normal (●), athymic nude (○), and immunocompetent mice. At various times after implantation mice were sacrificed, the tumor-bearing kidney was exteriorized, and xenograft size was measured grossly under a dissecting microscope equipped with an ocular micrometer. Change in xenograft size was calculated as the difference between xenograft size measured on the implant day and the assay day in the same mouse. Each value represents the mean change in xenograft size ± the range evaluated in two (A and B) or four (C) replicate mice.

third weeks after implantation (Fig. 1). These gross characteristics were never seen in the xenografts implanted in the immunocompetent mice. In fact the margins of the xenograft became very undefined and hazy during the second week after implantation in immunocompetent mice, making it difficult to measure xenograft size.

To assess the role of the host versus xenograft immune response in preventing tumor growth in immunocompetent mice, we examined the histopathological changes induced by the xenografts described in Chart 2B in immunocompetent mice and compared them to the changes induced by the same tumor in nude mice. As shown in Fig. 2 (Fig. 2A, a, c, e, and g) in immunocompetent mice, infiltration of the tumor with mononuclear cells and neutrophils began on day 3, became very heavy on day 6, and resulted in complete rejection of the tumor by day 9 after implantation. Tumor cells were never evident under the kidney capsule of immunocompetent mice during the second and third weeks after implantation. Mononuclear cells, fibro-
could not be determined by macroscopic measurement alone. This event of tumor rejection and laying down of scar tissue in immunocompetent mice was not accompanied by a consistent increase or decrease in xenograft size, and thus could not be determined by macroscopic measurement alone (Chart 2). In contrast in nude mice there was no infiltration of tumor by mononuclear cells or neutrophils on day 3, some infiltration of the tumor by mononuclear cells occurred on day 6, but there was no evidence of tumor rejection on day 9 after implantation (Fig. 2). Tumor expanded in the subrenal capsule space of nude mice during the second and third weeks after implantation, and tumor cells undergoing mitosis were frequently seen during this interval (Fig. 2).

Several other fresh surgical explants of human tumors were implanted as first transplant generation xenografts under the kidney capsule of normal and nude mice and were examined 6 days after implantation for amount of tumor in the xenograft and change in xenograft size. The results in Table 1 indicate that there was no correlation between amount of tumor in the xenograft and change in xenograft size during the first 6 days after implantation. Decreases as well as increases in xenograft size were found where the major portion of the subcapsular implant space was occupied by viable tumor. Changes in xenograft size were quite small, the largest being an increase of 0.4 mm in average diameter of the xenograft. In all cases the amount of tumor in the xenograft was markedly less in immunocompetent mice than it was in nude mice. In immunocompetent mice there was significant immunological infiltration around the tumor and evidence of tumor necrosis in all ten cases. In five of ten cases tumor had been completely eliminated from the subcapsular space in immunocompetent mice. In contrast in nude mice the major portion of the implant space was occupied by viable tumor cells, there was no evidence of tumor necrosis, and there was only sparse infiltration by mononuclear cells in ten of ten cases.

The amount of tumor in human colon cancer xenografts was compared to the macroscopic measurement of change in xenograft size at various times following implantation of tumor pieces under the kidney capsule of normal mice, nude mice, and mice immunosuppressed by alternative means, namely, (a) thymectomy followed by lethal irradiation and bone marrow reconstitution (TIB mice), or (b) daily treatment with cyclosporine (60 mg/kg/day) (Table 2). As described above, tumor was largely eliminated from the subrenal capsule space of immunocompetent mice by day 6 after implantation. In contrast the major portion of the implant site was occupied by tumor in nude mice, TIB mice, and cyclosporine-treated mice at day 6. During the second week after implantation tumor was completely eliminated from the subrenal capsule implant site in immunocompetent mice. However, tumor persisted and grew within the subcapsular space of immunosuppressed mice. TIB and cyclosporine-treated mice were comparable to nude mice in supporting the long-term persistence and growth of human tumor xenografts (Table 2). In fact in a separate series of studies which will be reported elsewhere, human tumor was still present and expanding in the subcapsular space of cyclosporine-treated mice 35 days after implantation.

**DISCUSSION**

The results of this study indicated that human tumors implanted as xenografts under the kidney capsule of immunocompetent mice induced significant infiltration of murine host defense elements into the subcapsular space, which resulted in immunological rejection of the tumor during the first week after implantation. This has been a consistent finding in over 12 tumors which we have studied and has not been due to a problem in tissue selection, since the same tumors thrived with no evidence of immunological rejection when they were implanted under the kidney capsule of nude mice. These results are in agreement with the studies of Edelstein et al. (3). However, recent studies by Aamdal et al. (7) have described significant growth of human tumor xenografts under the kidney capsules of both immunocompetent mice and nude mice in 6 days, leading these investigators to advocate use of immunocompetent mice in place of nude mice and a 6-day assay time frame for tumor chemosensitivity testing. The discrepancy between these results and those described both in this report and by Edelstein et al. (3) are most likely due to the fact that the studies carried out by Aamdal et al. (7) utilized fast-growing serially transplanted human tumor cell lines rather than freshly resected first transplant generation human tumors. We have found significant growth of fast-growing tumor cell lines by day 4 after implantation under the kidney capsule of immunocompetent mice, and have evaluated chemosensitivity within this time frame (13). However, these tumors were also heavily infiltrated with host immune cells by day 6 after implantation, preventing further xenograft growth. In our experience all cases of freshly resected human tumors implanted as first transplant generation xenografts have been slow growing.
compared to tumor cell lines, and have been immunologically rejected in immunocompetent mice before the development of significant tumor growth.

Other investigators have used immunocompetent mice as hosts for subrenal capsule implants of human tumors as a model for testing the chemosensitivity of these tumors during the first week after implantation (4–6, 9). In such a model the target of a chemotherapeutic agent could be either the human tumor component, the murine host defense component, or both. Drug toxicity to either of these components could result in reduction in xenograft size, which would complicate interpretation of tumor chemosensitivity. Levi et al. (5) have attempted to circumvent this problem by assaying 4 days rather than 6 days after implantation, and by evaluating each tumor-bearing kidney histologically. Although there is less immunological infiltrate in a 4-day assay, in this model there is no way of knowing the amount of tumor in each xenograft until the assay has been completed and the tumor-bearing kidneys have been examined histologically. Not knowing the amount or the integrity of tumor in the xenograft before drug treatment could complicate interpretation of tumor chemosensitivity in this model. Also, under the best of circumstances slow-growing human tumors would persist but not necessarily grow in a 4-day assay, and 4 days may not be long enough to observe the cytoreductive effects of certain drugs against such tumors.

Substitution of nude mice for immunocompetent mice eliminated infiltrating murine host defense components responsible for xenograft rejection. However, even in nude mice, we found that in most cases there were elements in addition to tumor making up the xenograft. Examples of such elements were human stroma, phenotypic products from the tumor such as mucin, vascular tissue, and some murine lymphoreticular cells and stroma. These contaminating noncancerous elements would complicate interpretation of chemosensitivity in a short-term assay and underscore the need for careful selection of tumor tissue for implantation. We have found histological examination of frozen sections from the tumor to be implanted very helpful in selecting areas of the tumor which were highly enriched in viable tumor cells. We have also found that allowing the tumor to grow for 2 weeks under the kidney capsule of immunodeficient mice resulted in a marked expansion in the amount of tumor within the subcapsular space, which significantly reduced contaminating noncancerous tissue.

Persistence and growth of human tumors under the kidney capsule of immunodeficient mice for 2 weeks provides a model in which the chemosensitivity of human tumors can be tested in vivo in a pharmacologically active system against growing solid tumors. Growth can be verified by examining the tumor-bearing kidney approximately 2 weeks after implantation during a second-look laparotomy. The mice tolerate this second surgical procedure quite well with less than 5% mortality. The criteria which we have established to grossly identify functioning and growing tumor under the kidney capsule during this second-look operation are, (a) increase in xenograft breadth, (b) increase in xenograft depth, (c) neovascularization of the xenograft, and (d) clear xenograft margins (Fig. 1). Histological evidence of tumor has been found in 100% of the cases in which these criteria have been satisfied, and these criteria have been satisfied in approximately 80% of the immunodeficient mice implanted with tumor. The remaining 20% presumably received noncancerous tissue, cancerous tissue which was not viable, or cancerous tissue which would not grow in the subrenal capsule space. The tumor take rate has been considerably lower than 80% in necrotic tumors or in tumors which were grossly interspersed with non-neoplastic tissue.

Nude mice were not optimal as immunoincompetent hosts for a subrenal capsule chemosensitivity assay, because they were not always available in large numbers from suppliers, and fastidious in their care and housing requirements. The results of this study indicated that the TIB or cyclosporine-treated conventional mice supported long-term persistence and growth of human tumor xenografts under the kidney capsule, and could be used as an alternative to nude mice in a long-term subrenal capsule chemosensitivity assay, providing a considerable saving in cost. Steel et al. (14) have carried out extensive tumor chemosensitivity studies in TIB mice bearing s.c. implants of human tumors. These mice tolerated chemotherapy, but tumor take rate was reduced when compared to take rate in studies where tumors were implanted in the subrenal capsule space of immunosuppressed mice. We have tested the chemosensitivity of carcinoma xenografts in the subrenal capsule space of cyclosporine-treated mice and have found the tumor chemosensitivity to be similar to that obtained in nude mice.4

The subrenal capsule space of mice is a suitable environment for growing and studying human solid tumors. The rich vascular bed in that environment bathes the tumor in nutrients, resulting in high tumor take rates (1, 8). Limiting factors for growing tumors in this environment such as host versus xenograft response and proper selection of tumor tissue can be overcome, respectively, by using appropriately immunosuppressed mice and by examining frozen sections of tumor tissue for guidance to areas of the tumor rich in viable tumor cells. Previous studies
by our group (13, 15) and by Maenpaa et al. (16) have demonstrated accurate prediction of chemosensitivity against fast-growing experimental solid tumors using this assay. However, the usefulness of this assay for predicting chemosensitivity of human tumors remains to be determined. Pharmacological questions pertaining to timing the initiation and duration of drug treatment and to measuring the duration of drug effect against growing tumors still need to be worked out. The biological problem of tumor heterogeneity which could potentially lead to a lack in uniformity of drug effect across a tumor specimen needs to be assessed. In spite of the need for further development, carrying out the SRC in immunosuppressed mice over a 3-week time period stands as a unique in vivo model in which human solid tumors can be reliably grown in a relatively short period of time, and in which chemosensitivity testing can be carried out against tumors whose growth has been verified before drug exposure.

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REFERENCES


Fig. 1. Low power macroscopic picture of a human colon cancer xenograft 18 days after implantation under the kidney capsule of a nude mouse. Note well-defined margins of xenograft and network of prominent capsular vessels providing vascular support for the tumor. × 12.
Fig. 2. Human colon adenocarcinoma (1) xenografts implanted under the capsule (C) of the mouse kidney (K). Stained with H & E. A, Unimmunocompetent mouse at day 3 after implantation. Note absence of neoplastic changes and normalcy of renal cortex. X 40. B, Immunocompetent mouse at day 6 after implantation. Note presence of neoplastic changes and normalcy of renal cortex. X 40. C, Unimmunocompetent mouse at day 9 after implantation. Note intense mononuclear cell infiltrate with absence of tumor in renal cortex. X 40. D, Immunocompetent mouse at day 12 after implantation. Note complete occupation of xenograft by tumor and bulging of kidney capsule due to growing tumor. X 40.
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