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Human Lung Tumor Growth Established in the Lung and Subcutaneous Tissue of Mice with Severe Combined Immunodeficiency[1]

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ABSTRACT

We report here that a mouse mutant (C.B-17 scid) which lacks functional B- and T-lymphocytes can be used to propagate a human lung tumor. The heterotransplanted tumor cells generated palpable s.c. tumors by 18 days in 100% of the mice inoculated s.c. with >4 × 10^6 cells. All tumors grew progressively with no sign of regression. A portion of the scid mice given injections i.v. of the human lung tumor cells developed multiple tumor nodules in the lung by 15 weeks after the inoculation of tumor cells. The tumor nodules were shown by karyotype analysis to be human cells, and the histopathology of the tumor nodules revealed a pattern of growth that was consistent with that of the original tumor. The human lung tumor used in the study expresses an M_r 160,000 cell surface glycoprotein that has been shown to occur on a large proportion of human lung tumors and tumor cell lines. A monoclonal antibody specific for M_r 160,000 glycoprotein was used to demonstrate that this tumor-associated antigen is stably expressed by the s.c. tumors and the lung tumor nodules in the scid mice. The mutant mice with this severe combined immunodeficiency represent a new and viable model for propagating human tumors and for evaluating the efficacy of novel drug delivery protocols in the treatment of human cancer.

INTRODUCTION

Homozygous *nu/nu* mice have been used extensively for heterotransplantation of human tumors (1, 2). The growth of both surgically obtained tumors and human tumor cell lines has been studied in this mouse model (3, 4). Approximately 28% of the tumors grew in nude mice following the s.c. inoculation of surgically obtained tumors (3) or human tumor cell lines (4).

In order to increase the percentage of tumors that are able to be propagated in nude mice, additional immunodeficiencies were induced both artificially and genetically in nude mice. The NIH-2 mouse, for example, has a *nu* gene plus an X-linked gene for B-cell anergy (causing immunodeficiency). A small minority of tumors were found which grew in NIH-2 mice but not in nude mice. Most of these tumors grew in the s.c. tissue of the recipient mice.

A potentially ideal mouse for heterotransplantation of human tumors is the scid[4] mouse, described by Bosma *et al.* (5). They identified an autosomal mutation in the C.B-17 inbred strain from their specific pathogen-free mouse colony that severely impaired lymphopoiesis. Scid mice were found to have lymphopenia, a rudimentary thymic medulla without cortex, relatively empty splenic follicles and lymph nodes, and undeveloped bronchial and gastrointestinal lymphocytic foci (6). No pre-B- or B-cells were identified, but a few apparently nonfunctional T-cells were found in thymus and spleen and these were found to be highly disposed to neoplasia (thymic T-cell lymphomas were observed in 41 of 269 mice). Cells of myeloid lineage are normal; so also are natural killer cells (7) and the antigen-presenting cells (8). Reconstitution experiments have suggested that the microenvironment of the C.B-17 scid mouse is conducive to lymphocyte differentiation, and that scid mutation affects the differentiation of a pluripotent stem cell or a lymphoid-restricted stem cell into B- and T-lymphocytes (9). More recently it was established that the B- and T-cells of the scid mouse lack functional rearrangement of the genes coding for the antigen-specific receptors and that the scid mutation *per se* may adversely affect the recombinase system which is responsible for catalyzing the assembly of antigen-specific receptor genes in the developing lymphocytes (10).

The purpose of this study was to determine whether or not the scid mouse is a suitable model for the propagation of human tumors. We report here that a human lung tumor cell line A549 (11) can be successfully propagated in scid mice without any alteration in the tumor's karyotype, morphology, or expression of a tumor-associated antigen. Moreover tumor growth was observed in the lung of scid mice following the i.v. inoculation of tumor cells making this immunodeficient mouse a particularly attractive model with which to evaluate the efficacy of new therapeutic approaches such as immunospecific drug delivery protocols.

MATERIALS AND METHODS

Cells. The A549 tumor cell line was derived from a human alveolar lung tumor (11). These cells were maintained in culture at 37°C 5% CO_2 in RPMI 1640 medium supplemented with 10% fetal calf serum. Culture medium was changed two to three times a week. Cells were harvested from actively growing cultures by gentle scraping of the cultures to remove the attached cells from the plastic culture flasks. Cells were washed, and the cell suspension was adjusted to give a concentration of 1 × 10^7 cells/ml for s.c. and 2–4 × 10^6 cells/ml for i.v. injection. Viability of the cells was determined by trypan blue dye exclusion.

Animals, Breeding, and Handling. Two male and four female mice of the C.B-17 inbred strain (BALB/c-C57B1/Ka-Igh-1^b), all heterozygous for the scid gene were a gift from Dr. Mel Bosma, Institute for Cancer Research, Philadelphia, PA). These mice were set up as breeding pairs and their progeny tested for Ig deficiency by a radioimmunoassay of their sera for immunoglobulin. Mice homozygous for the scid gene have <1 μg of immunoglobulin/ml of serum. The homozygous scid mice were then set up as monogamous breeding pairs and the line propagated as brother × sister matings. To minimize genetic drift between C.B-17 scid and normal C.B-17, offspring of the fifth generation homozygous scid breeders were backcrossed to C.B-17 and the process repeated. The scid mice were housed in polycarbonate microisolater cages (Lab Products, Maywood NJ) on hardwood shavings. Water, acidified with 2.0 N HCl to a pH of 2.5–2.8 and a sterilizable mouse diet (Teklad Mills, Windfield, IA) were supplied *ad libitum*. All caging, food, water, and bedding were autoclaved before use. The animal cages were maintained within a high-efficiency particulate air-filtered laminar flow hood. Automatic timers were installed to provide a 12-h light/dark cycle. Personnel working with scid mice wore a face mask, head cover, disposable overshoes, a sterile surgical gown, and sterile gloves. Aseptic procedures were routinely utilized. The average litter

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[4] The abbreviations used are: scid, severe combined immunodeficiency; gp160, cell surface glycoprotein with an estimated molecular weight of 160,000; SRBC, sheep RBC.

size of the homozygous scid breeders in our colony was 3.5. Production index is relatively low since a breeding female will normally produce only about three litters.

Tumor Inoculation. Adult male and female scid mice (>6 weeks of age) were used for this study. Mice were given injections either s.c. on the lateral chest wall of $0.4–1 \times 10^7$ A549 cells or i.v. of $0.1–1 \times 10^7$ cells in a volume of 0.1–0.5 ml of 0.9% saline solution.

Tumor Histopathology. Excised tumors were fixed in 10% buffered formalin, sectioned, and stained with hematoxylin and eosin.

Monoclonal Antibody. A monoclonal antibody (5E8) was produced by fusing spleen cells from BALB/c mice immunized with surgically obtained human squamous cell carcinoma of the lung with mouse myeloma line X63-Ag8.653 (12). Using an immunocytoadherence test, this antibody was shown to bind to established lung tumor cell lines derived from an adeno (PC-3), a poorly differentiated adeno (PC-9), a squamous (PC-10), and an alveolar cell (A549) carcinoma, and two fresh tumors (squamous and poorly differentiated adenocarcinomas) obtained surgically, but not to most normal human lung tissue (12).

Tumor-associated Antigen. A plasma membrane-associated gp 160 has been identified in human lung tumors by using the monoclonal antibody 5E8 (13). This macromolecule is found on both lung tumor cell lines and on surgically excised fresh lung tumor tissue. Cell suspensions of A549 cells from portions of the tumor removed from scid mice were tested for the presence of gp 160 either directly or after passing in culture medium (RPMI 1640 supplemented with 10% fetal calf serum) using an immunocytoadherence assay.

Immunocytoadherent Assay. Target cells (A549) were washed three times with serum-free medium and resuspended to a concentration of 4×10^6/ml. Thirty μl of target cell suspension (1.2×10^5 cells) and 40 μl of either monoclonal antibody 5E8 (6.4 μg/ml) or normal mouse serum (10 μg/ml) were placed into conical plastic 1.5-ml centrifuge tubes (American Scientific) in duplicate and incubated for 20 min at room temperature. The cells were washed three times and resuspended. Thirty μl of a 1% suspension of SRBC to which affinity-purified rabbit anti-mouse Ig was covalently linked via a heterobifunctional reagent *N*-succinimidyl 3-(2-pyridyldithio)propionate (R α-mouse Ig-coupled SRBC) (14), were added to the resuspended cell suspension. The tubes were centrifuged ($400 \times g$) for 3 min and maintained on ice until observation. The cell pellet was resuspended and examined under a microscope.

Positive test (*i.e.*, where the 5E8 antibody has reacted with target cells) is manifested by the adherence of R α-mouse Ig-coupled SRBC to the tumor target cells, thus forming rosettes. The percentage of target cells with five or more adherent R α-mouse Ig-coupled SRBC (*i.e.*, rosette) was determined and reported as the average value (percent) of four determinations. Normal mouse serum was the negative control in this assay.

Cytogenetics. Cells obtained from tumors growing in scid mice were passaged once *in vitro* prior to karyotyping. The cells were harvested by scraping and then incubated with Colcemid for 2 h followed by hypotonic lysis (0.075 M KCl) for 20 min. After fixation with freshly prepared methanol:acetic acid (3:1), slides were prepared. Slides were then incubated at 60°C overnight, and stained with Wright stain for 1–2 min.

RESULTS

Growth of Human Tumor in scid Mice. Twenty-seven scid mice were given injections either s.c. or i.v. with the human lung tumor cell line A549. This particular human tumor cell line was selected since it expresses a cell surface gp160 which has been shown to be associated with a large proportion of lung tumor biopsies tested including squamous cell carcinomas, adenocaracinomas, large cell carcinoma, and alveolar cell carcinomas (12, 13). The results presented in Table 1 establish that the immunodeficient scid mouse can be used to propagate a human lung tumor. All 14 of the scid mice inoculated s.c. with tumor cells had tumor nodules at the site of injection 18 days after tumor inoculation. The tumor nodules which measured 0.5–1 cm at 1 month grew progressively for 3 months, reaching a diameter of 3 cm. No evidence of spontaneous regression was observed in any of the 14 tumor-bearing mice during this observation period. Postmortem examination with detailed inspection of lung, liver, kidneys, and spleen showed no evidence of tumor growth outside of the s.c. site of inoculation. Histological examination of lungs in three mice and liver, kidneys, and spleen in one mouse showed no evidence of tumor.

Table 1 *Growth of human lung tumor in scid mice following s.c. or i.v. inoculation of A549 cells*

Route of tumor inoculation	No. of tumor cells injected[a]	Mice with tumor / Mice inoculated[b]	No. of mice with tumor growth in lung
s.c.	$4–10 \times 10^6$	14/14	0
i.v.	$1–10 \times 10^6$	5/10	5

[a] Mice were given injections of single cell suspensions of A549 human lung tumor cells.

[b] s.c. tumor nodules observed in all mice 18 days after a single s.c. inoculation of tumor cells. Mice were sacrificed at varying intervals, and viscera were examined for evidence of tumor growth both grossly and microscopically (no microscopic examination in four mice).

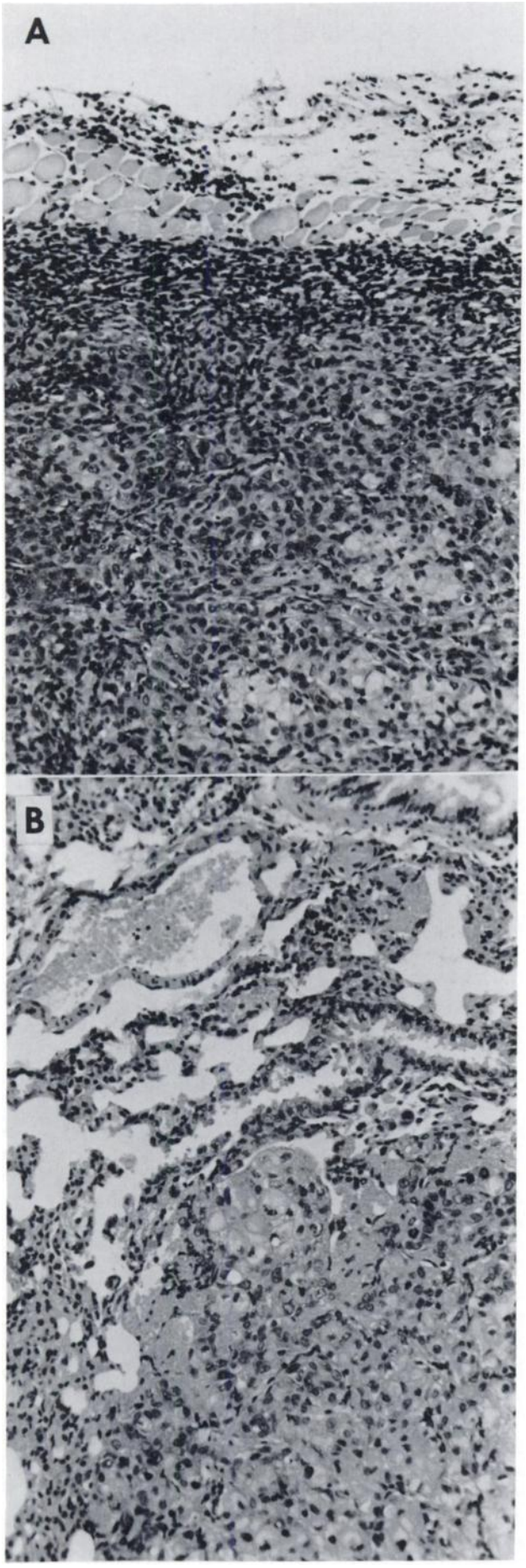
Ten mice were inoculated with the lung tumor cells i.v. and sacrificed between 8 and 29 weeks after tumor inoculation. Each mouse was examined for gross or microscopic evidence of tumor growth. Gross evidence of tumor was observed in the lungs of five mice. In each case multiple tumor nodules were noted in both left and right lungs, and the presence of tumor was confirmed histologically (in all but one mouse). In this one mouse in which no histological evidence for tumor could be found, the presence of tumor was confirmed by cultivation of the lung tissue from this mouse. Tumor growth in the lung was restricted primarily to the periphery of the lungs. Close inspection either grossly and/or microscopically revealed no evidence of tumor growth in five remaining mice and in none of the 10 mice was there any evidence of tumor growth outside of the lung in spite of a close inspection of liver, spleen, kidney, and adrenal gland (including microscopic examination in four).

Subpassage of Tumor *in Vitro and in Vivo*. An attempt was now made to determine whether or not the tumors growing in the scid mice could be removed and cultivated either in tissue culture or retransplanted to uninoculated as well as previously inoculated scid mice. It was first established that either the s.c. growing tumors or the tumor nodules from the lung could be propagated *in vitro*. The tumor cells which grew out of these cultures had the same morphology and surface phenotype (*i.e.*, gp160 positive) as the parental A549 cells.

Tumor cells derived from the s.c. tumor nodules and the lung tumor nodules (first passage) were found to give rise to tumor nodules (second passage) at the site of injection when reinoculated s.c. and again in the lung when inoculated i.v.

In another group of mice the lung tumor was first surgically excised and subsequently tumor cells were reinjected into an s.c. site which was the same or different from that of the previous xenograft. Second passage tumor growth was observed in mice given the tumor in the same or different s.c. site.

Histopathology. Two key points were noted with the histopathology of the s.c. growing tumor nodules and the tumor growing in the lung: (*a*) the morphology of the tumor growing s.c. was identical to tumors growing in the parenchyma of the lung (Fig. 1); (*b*) the tumor propagated *in vivo* had sheets of tumor cells with excentric nuclei and vacuolated cytoplasm. This morphology is consistent with poorly differentiated adenocarcinoma and similar to that reported previously for this tumor (11). This pattern was preserved after two passages s.c. and after one passage s.c. followed by an i.v. tumor inoculation



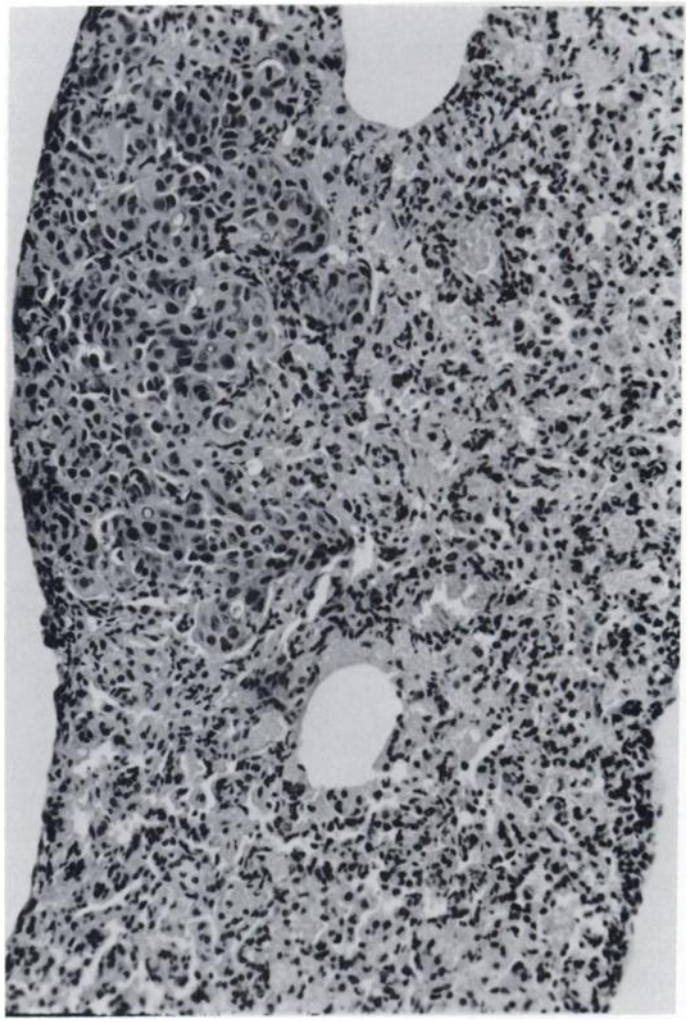


Fig. 2. Photomicrograph of the tumor growing in the lungs of an scid mouse following i.v. inoculation of 1.5×10^6 A549-sc/L (A549 after one s.c. and one lung passage). Sheets of anaplastic carcinoma cells with pleomorphic nuclei are present which is consistent with a more poorly differentiated large cell carcinoma. Hematoxylin and eosin, × 240.

and growth in the lung. However, as shown in Fig. 2 the tumor morphology was altered to resemble that of a more poorly differentiated large cell carcinoma after three passages in an scid mouse (one s.c. growth followed by two subsequent growth periods in the lung). After multiple passages the cells appeared more squamoid with pleomophic nuclei.

Chromosome Analysis. In order to establish that the tumors observed in the scid mice were of human origin and not a spontaneous murine tumor or possibly a mouse/human hybrid the tumor cells were karyotyped before and after passage in the scid mouse. Chromosomes were identified to be human in origin (no mouse chromosomes were seen in any of the preparations). The number of chromosomes ranged from 63–74, and this variability in chromosome number was seen in both the *in vitro* and *in vivo* passaged cells. The results of these studies indicated that the tumors observed in the scid mice were of human origin

Fig. 1. *A*, photomicrograph of the tumor grown s.c. in scid mouse following s.c. inoculation of 10×10^6 A549 cells. Anaplastic carcinoma cells arranged in sheets with vacuolated cytoplasm are present. This histological pattern was observed after two s.c. passages of the tumor. *B*, photomicrograph of the tumor induced in the lungs following i.v. inoculation of 1×10^6 A549 cells. Anaplastic carcinoma cells arranged in sheets are seen infiltrating the lung parenchyma. This morphology is consistent with that of a poorly differentiated adenocarcinoma and is similar to that which was reported previously for the tumor (11). Hematoxylin and eosin, × 240.

and that no change in the karyotype occurred after the passages of the tumor *in vivo*.

Effect of *in Vivo* Passage on the Expression of Tumor-associated Antigen. Tumors propagated either *in vivo* or *in vitro* may alter their expression of one or more cell surface antigens. In this regard we were interested to know whether or not the growth of the A549 cells in the scid mice would alter the expression of a cell surface glycoprotein (gp160) which has been shown to be associated with a wide variety of human lung tumors including A549 cells (12, 13). This question was addressed by assaying A549 cells for the presence of gp160 after two or three passages in scid mice. The presence of the gp160 is determined using a monoclonal antibody 5E8 which is specific for this antigen. The binding of 5E8 to gp160 is detected by the addition of indicator sheep RBC to which rabbit anti-mouse antibody has been covalently coupled. The results presented in Table 2 indicate that the tumor-associated antigen is stably expressed after two passages s.c. or after a single passage s.c. and a subsequent passage in the lung. A small decrease in the percentage of cells that were positive for gp160 was seen with tumor cells that had been passaged once s.c. and twice in the lung.

DISCUSSION

We report here that a human lung tumor can be successfully propagated in mice homozygous for a mutation which renders them deficient for both B- and T-lymphocytes. This severe combined immunodeficient strain (C.B-17 scid) was first described in 1983 (5) and has since been used as a model to study lymphocyte function and differentiation at the cellular (8, 15, 16) and molecular level (10).

The ability of the C.B-17 scid to support the growth of a human tumor establishes this strain as a new model for investigating human tumor growth *in vivo*. Specifically this mutant represents a viable resource for evaluating novel therapeutic approaches for the treatment of cancer. One such approach that is being investigated by our laboratory is to utilize monoclonal antibodies (specific for a cell surface tumor-associated antigen) to target the delivery of cytotoxic drugs to human lung tumors. In this system, one would like to test the efficacy of a therapeutic protocol in an animal model in which the human lung tumor is growing in the lung of the recipient animal. The observation reported here that at least a portion of the scid mice inoculated with tumor cells i.v. ultimately developed tumor growth in the lung is particularly attractive in this regard. We have also observed that the tumor grows in the lung when tumor cells are injected directly into the lung of the scid mice. Equally important in such an animal model is that the tumor phenotype remains stable during its propagation *in vivo*. We have observed that little or no alteration in tumor morphology or karyotype occurs during one or two growth cycles *in vivo*, but most importantly, it has been established that the target of our monoclonal antibody-directed drug delivery (an M_r 160,000 glycoprotein) is maintained on the tumor cell surface for at least three passages in the scid mouse (one passage s.c. and two growth cycles in the lung). These findings warrant that the C.B-17 scid mouse is a new and attractive model with which to evaluate our immunospecific drug delivery system using human lung tumors.

Another group of investigators has observed that the C.B-17 scid mouse supports the growth of a variety of different human tumors. The other tumor types shown to grow in scid mice include adenocarcinomas of the bladder, prostate, and pancreas, osteosarcomas, a testicular tumor, and Wilms' tumor.[5] All of these tumors were transplanted directly following surgical biopsies. Retinoblastomas were found to grow in the anterior chamber of the eye of the scid mouse.[6]

Many of the tumors that grow in the scid mice will also grow in nude mice. We have determined that the A549 tumor will grow in irradiated nude mice (500 R/mouse). However, the scid mouse in some instances represents a much better model for propagating human tumors than does the nude mouse. This is particularly true when investigating immunospecific drug delivery protocols. One obvious advantage of the scid mouse is that these mice, as opposed to nude mice, have little or no immunoglobulin in their serum or interstitial fluid. This makes the task of determining the *in vivo* localization of anti-tumor antibody quite simple using i.v. injected unlabeled antibody. The localization of this antibody can be monitored easily by staining frozen tissue sections using fluorescein-labeled rabbit anti-mouse immunoglobulin. Another advantage of the scid mouse is the inability of these mice to respond to either thymic-dependent or -independent antigens. Thus, one does not have to contend with the possibility of the host humoral immune response to ligands (such as dextran) which are used to link antibodies and the cytotoxic drugs. Also, the scid mouse does not have the potential problem of antibodies that are cross-reactive with determinants on the tumor or on the targeting antibody. Such antibodies may be present in the sera of nude mice prior to tumor inoculation or administration of antibody-drug conjugates or they may be induced subsequently by thymic-independent determinants present on the tumor. Either host anti-tumor antibody or anti-immunoglobulin could interfere with the ability of antibody-drug conjugates to localize to their tumor targets.

The C.B-17 scid mice are currently being introduced into the mouse colony at The Jackson Laboratory, Bar Harbor, ME. It is expected that these mice will be made available to investigators by the Fall of 1987. The mice can be maintained and successfully propagated under reasonably aseptic procedures and standard breeding protocols (see "Materials and Methods"). Two limitations that we have encountered in the propagation of these mice are the relatively small litter size and the fact that a female homozygous scid mouse will normally produce only three litters.

Table 2 *Effect of growth in scid mice upon expression of tumor-associated antigen (gp160)*

Tumor	No. of passages *in vivo*	Site of tumor growth	% cells with gp160[a]	
			5E8[b]	Normal mouse serum
A549	0		80.0	0
A549/A	2	S.c./s.c.[c]	85.8	0
A549/B	2	S.c./L[d]	89.8	0
A549/C	3	S.c./L/L	62.3	0

[a] The presence of gp160 was established by an immunocytoadherence assay. See "Materials and Methods" for details.

[b] The monoclonal antibody 5E8 binds to a cell surface glycoprotein with a molecular weight of 160,000 (13). In each assay 0.25 μg of antibody were used.

[c] S.c./s.c. indicates two passages s.c. L indicates growth in the lung; s.c./L indicates one passage s.c. and one passage i.v. with growth in lung. S.c./L/L indicates one passage s.c. with two passages i.v. with growth in lung.

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[5] B. L. Gallie and R. A. Phillips, Sick Children's Hospital, Toronto, Ontario, Canada and M. J. Bosma, Institute for Cancer Research, Philadelphia, PA, personal communication.

[6] R. A. Phillips, personal communication.

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REFERENCES

1. Fogh, J., and Trempe, G. New human tumor cell lines. *In:* J. Fogh (ed.), Human Tumor Cells *in Vitro*, pp. 115–159. New York: Plenum Publishing Corp., 1975.
2. Fogh, J., and Hajdu, S. I. The nude mouse as a diagnostic tool in human tumor cell research. J. Cell Biol., *67:* 117a, 1975.
3. Fogh, J., Dracopoli, N., Loveless, J. D., and Fogh, H. Prog. Clin. Biol. Res., *89:* 191–223, 1982.
4. Fogh, J., Fogh, J. M., and Orfeo, T. One hundred and twenty-seven cultured human tumor cell lines producing tumors in nude mice. J. Natl. Cancer Inst., *59:* 221–225, 1977.
5. Bosma, G. C., Custer, R. P., and Bosma, J. M. A severe combined immunodeficiency mutation in the mouse. Nature (Lond.), *301:* 527–530, 1983.
6. Custer, R. P., Bosma, G. C., and Bosma, M. J. Severe combined immunodeficiency (SCID) in the mouse; pathology, reconstitution, neoplasms. Am. J. Pathol., *120:* 464–477, 1985.
7. Dorshkind, K., Pollack, S. B., Bosma, M. J., and Phillips, R. A. Natural killer (NK) cells are present in mice with severe combined immunodeficiency (scid). J. Immunol., *134:* 3798–3801, 1985.
8. Czitrom, A. A., Edwards, S., Phillips, R. A., Bosma, M. J., Marrack, P., and Kappler, J. W. The function of antigen-presenting cells in mice with severe combined immunodeficiency. J. Immunol., *134:* 2276–2280, 1985.
9. Dorshkind, K., Keller, G. M., Phillips, R. A., Miller, R. G., Bosma, G. C., O'Toole, M., and Bosma, M. J. Functional status of cells from lymphoid and myeloid tissues in mice with severe combined immunodeficiency disease. J. Immunol., *132:* 1804–1808, 1984.
10. Schuler, W., Weiler, I. J., Schuler, A., Phillips, R. A., Rosenberg, N., Mak, T. W., Kearney, J. F., Perry, R. P., and Bosma, M. J. Rearrangement of antigen receptor genes is defective in mice with severe combined immune deficiency. Cell, *46:* 963–972, 1986.
11. Lieber, M., Smith, B., Szakal, A., Nelson-Rees, W., and Todaro, G. Continuous tumor-cell line from a human lung carcinoma with properties of type II avelar epithelial cells. Int. J. Cancer, *17:* 62–70, 1976.
12. Saji, S., Zylstra, S., Schepart, B. S., Ghosh, S. K., Jou, Y-H., Takita, H., and Bankert, R. B. Monoclonal antibodies specific for two different histological types of human lung carcinoma. Hybridoma, *3:* 119–129, 1984.
13. Zylstra, S., Chen, F-A., Ghosh, S. K., Repasky, E. A., Rao, U., Takita, H., and Bankert, R. B. Membrane-associated glycoprotein (gp160) identified on human lung tumors by a monoclonal antibody. Cancer Res., *46:* 6446–6451, 1986.
14. Jou, Y-H., Mazzaferro, P. K., Mayers, G. L., and Bankert, R. B. Methods for the attachment of haptens and proteins to erythrocytes. *In:* J. J. Langone and H. Van Vunakis (eds.), Methods in Enzymology, pp. 257–276. New York: Academic Press, Inc., 1983.
15. Bancroft, G. J., Bosma, M. J., Bosma, G. C., and Unanue, E. R. Regulation of a macrophage Ia expression in mice with severe combined immunodeficiency: induction of Ia expression by a T cell-independent mechanism. J. Immunol., *137:* 4–9, 1986.
16. Fulop, G. M., and Phillips, R. A. Full reconstitution of the immune deficiency in scid mice with normal stem cells required low-dose irradiation of the recipients. J. Immunol., *136:* 4438–4443, 1986.