Stroma Is Critical for Preventing or Permitting Immunological Destruction of Antigenic Cancer Cells

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Summary

Inoculated immunogenic cancer cells after initial growth are potentially rejected by specific host immunity; however, the outcome of the interaction between host and inoculated cancer cells is a function of multiple factors including the route of inoculation, the number of cells, the density of antigens on the injected cancer cells, and the state of the immune system of the host. In the present study, we have examined a different kind of variable: the stroma that inoculated tumor cells initially reside in. The impetus to examine this factor arises from observations that cancer cells from several lines inoculated as fragments of solid tumors often grow progressively, whereas the same number or more than 10-fold larger numbers of identical type cells injected as a suspension are rejected, even though fragments or suspended cells are both tumorigenic at the same doses in nude mice. In the present studies, we found that: (*a*) indeed, cancer cells inoculated as fragments were more tumorigenic than cancer cells in suspension; (*b*) the tumorigenicity of suspended cancer cells was increased by injection of the cells into polyurethane sponge implants; (*c*) cancer cells were more tumorigenic embedded in syngeneic stroma than in transgenic antigenic stroma expressing the K^{216} major histocompatibility complex class I antigen; and (*d*) antigenic, bone marrow–derived, stromal components (presumably passenger leukocytes) were sufficient to cause rejection of immunogenic but antigenically unrelated cancer. Together, these studies show that the stromal milieu helps determine the outcome of the complex interaction between host immunity and cancer cells and suggests a new mode of immunotherapy based upon targeting of antigenic leukocytes to the tumor site to serve as stimulators of immunological rejection of cancer.

Malignant cells of solid cancers invade surrounding normal tissues; thus, cancer cells[1] become embedded in a matrix of nonmalignant tissue consisting of vessels, sessile and migratory cells, and extracellular matrix, which together are termed "tumor stroma" (1). Tumor stroma seems to play a complex role in tumor growth. Cancers can invade and destroy the surrounding normal tissues, including stroma; however, tumor stroma is essential for providing blood supply and other factors that affect the growth of cancers. While some tumors use mainly preexisting surrounding tissues as stroma, other cancers induce formation of new stroma. Some cancers influence surrounding stroma by inducing expression of surface antigens on stromal cells (2). The interactions between cancer cells and stroma are poorly understood, but probably affect invasion and metastasis by malignant cells (for review see references 3 and 4) as well as infiltration and reactivity of immune and other cells of inflammation. The study of such stromal interactions may, therefore, reveal new targets for alternative therapeutic strategies against solid tumors. In the previous study, we show that while nonantigenic stroma may prevent effective immunological destruction of malignant cells, antigenic stroma can lead to rejection of cancer cells that are embedded in it.

Materials and Methods

Mice. 5-wk-old female C3H/HeN (mammary tumor virus-negative [MTV^-][2]) mice from colonies of germfree-derived, defined flora animals were purchased from the NCI Frederick Cancer Research Facility (Frederick, MD). The original stock of nude C3H mice was in its 23rd backcross generation when obtained from a colony of the Biology Division of the Oak Ridge National Laboratory (Oak Ridge, TN). After 1988, the nude mice of this strain were purchased from the NCI Frederick Cancer Research Facility.

[1] Carcinomas and sarcomas are referred to as malignancies or cancers, and cells derived from them as cancer cells or tumor cells, while the term tumor or tumorigenic describes the mass or growth produced by the cancer cells.

[2] *Abbreviations used in this paper:* MTV, mammary tumor virus; PRO, progressor; TBA, tumor-bearing animals.

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The derivation of the K^{216} transgenic C3H/HeN (MTV^-) mice has been described (5). Gene K^{216} was isolated from a genomic library of the 1591 RE (6) and is not identical but very similar to (three amino acid differences) a K^s recently cloned and sequenced (K. Hasenkrug and S. Nathenson, personal communication). The transgenic mice express the K^{216} MHC class I alloantigen with the same or similar tissue distribution as normal MHC class I antigens (5). Before use in experiments, transgenic mice were screened for expression of the K^{216} gene in PBL by flow cytofluorometry. All mice were kept in laminar air flow hoods and were fed sterilized food (Purina 5010 C; Purina, Inc., St. Louis, MO) and sterilized, acidified water.

Tumor Lines. The undifferentiated spindle cell cancer 1591-PRO (also designated 1591-PRO4L or 1591-VAR8 [7]) used in this study is one of the progressor variants observed in 5 of 100 animals that were challenged with fragments of the 1591 regressor tumor (8, 9). The 1591 regressor expresses in addition to K^k and D^k three other MHC class I genes: D^q, L^q, and K^{216}, which is similar to K^s, (6, 10, and K. Hasenkrug and S. Nathenson, personal communication). The 1591-PRO tumor expresses K^k and D^k MHC class I antigens but has lost all three MHC class I genes that are not of $H\text{-}2^k$ haplotype (7, 11). Fragments of the 1591 PRO tumor will grow progressively in ~80% of normal mice. This progressor variant when transfected with the K^{216} gene (TR3.3, designated K^{216} tumor or 1591-RE) is always rejected by normal C3H/HeN mice unless a rare variant arises with loss of the K^{216} gene (7). The 1591-MET is a highly malignant variant of the 1591 tumor that expresses K^k and D^k antigens but lacks all four previously described CTL-defined target antigens (11). 6132A-PRO (clone 2.2), 6118A-PRO, (Subline PRO1), and 6134A-PRO are progressor variants of recently derived UV-induced regressor tumors from C3H/HeN hosts (12, 13). Morison et al. (14) have reported that most skin tumors induced with UV in C3H mice are of epithelial origin. AG-104A is a spontaneous undifferentiated malignant tumor that developed in the subcutaneous tissue of an aged C3H/HeN mouse (13). The fibrosarcoma 3152-PRO was induced in a UV-irradiated C3H/HeN mouse by the subcutaneous injection of 3-methylcholanthrene in non-UV-exposed skin (15). All cancer cell lines were cultured in vitro in MEM containing 10% heat-inactivated FCS (CMEM).

Determination of Cells in Solid Tumor Fragments. The number of cells injected with tumor fragments in a full trocar was estimated by comparing the volume of tumor fragments within a trocar and the volume of a tumor cell. The volume of tumor fragments within a trocar was measured by determining the volume of medium displaced by the fragments loaded in a 13-gauge trocar (Becton Dickinson & Co., Mountain View, CA). The trocar load was expelled onto 3MM blotting paper (3mmChr; Whatman International Ltd., Maidstone, England) to remove the surrounding liquid, and the fragments were then immediately transferred with a forceps into an inverted tuberculin syringe used as volumetric vessel. The volume of fragments in one trocar load measured in six experiments (measuring five trocar loads for each line and four different cell lines) ranged from 27 ± 15 μl SD to 31 ± 16 μl SD (average, 29 ± 14 μl SD). The average volume of a tumor cell was determined by adding a large number of tissue culture cells ($1\text{–}2 \times 10^8$) to 1.5 ml of medium in a 2-ml pipette. The mean volume per tumor cell ranged from $1.95 \times 10^{-6} \pm 0.16 \times 10^{-6}$ μl SD to $2.15 \times 10^{-6} \pm 0.18 \times 10^{-6}$ μl SD for the four cell lines tested. This value lies within the average range of single cell volumes (5.2×10^{-7} to 1.4×10^{-5} μl) reported previously for animal cells (16). Using the experimental value for cultured single cancer cell volume of UV-induced cancer cell lines and the experimental value for the volume of tumor fragments injected with a trocar, the mean value of cancer cell number per trocar is $1.4 \times 10^7 \pm 0.7 \times 10^7$ tumor cells, which for convenience is referred to in the tables as 1.5×10^7 tumor cells per trocar.

Tumor Transplantation, Biopsy, and Readaptation to Culture and Enzymatic Digestion. Solid tumors were obtained from nude C3H mice that had been inoculated with cultured cells; ~1-mm³ fragments were transplanted subcutaneously with a trocar into anesthetized mice, either as full, 1/3, or 1/10 full trocar load. To confirm the 1591 lineage or K^{216} antigen expression of a tumor, fine needle biopsies were routinely obtained after 4–5 wk of tumor growth as described (5). Tumor biopsies were expelled into CMEM containing antibiotics (penicillin, streptomycin, and gentamycin) and cultured for 1 wk before flow cytofluorometric analysis of antigen expression. For some experiments, tumor fragments were digested enzymatically to generate single cell suspensions. Fragments were digested in RPMI containing 10 mg deoxyribonuclease, 100 mg collagenase (Sigma Chemical Co., St. Louis, MO), and 250 U hyaluronidase (Sigma Chemical Co.) for 1 h at room temperature. Digested cells were filtered through a nylon screen (60-μm mesh width) and the suspension was then washed four times with CMEM and counted before use in experiment.

Skin Transplantation. Ventral donor skin was applied to the dorsal thoracic wall of the recipient using the method of Billingham and Medawar (17). Bandages were removed on day 7, and grafts were scored daily until rejection (defined as loss of at least 80% of grafted tissue) or the end of the experiment.

Polyurethane Sponge Matrix Implants. A 0.50–0.75-cm³ polyurethane sponge (Future Foam Co., Chicago, IL) was passed through a skin incision anterior to the base of the tail and deposited subcutaneously in the interscapular region of anesthetized mice. Sterility is crucial since the sponge graft acts as a foreign body and will support bacterial growth.

Flow Cytometry Analysis. The mAbs CP28 (18) and CP154 are specific for the K^{216} MHC class I antigen and for a 1591 lineage-specific antigen, respectively. The other anti-MHC class I mAbs were gifts from Dr. Keiko Ozato and their specificities have been described (19). $0.5\text{–}1 \times 10^6$ cells were incubated with the specific antibodies for 30 min on ice and then washed twice with PBS containing 10% BSA and 0.1% sodium azide. Next, the cells were incubated with fluorescein-coupled goat anti–mouse immunoglobulin (Hyclone E-1081-A; Hyclone Laboratories, Logan, UT) for 30 min before analyzing them on either FACS IV (Becton Dickinson & Co.) or on EPICS-753 (Coulter Electronics, Hialeah, FL). The binding ratio was determined as the amount of fluorescence after staining with both antibodies divided by the amount of fluorescence after staining with the second antibody alone.

Results

Cancer Cells Are More Tumorigenic When Contained within a Stromal Matrix. Our studies were predicated on the initial observations that certain cancer cell lines capable of progressive growth when administered as solid tissue fragments ("progressor [PRO] tumors") were incapable of such growth when administered as suspensions of cells. For example, the UV-induced tumor lines 1591-PRO, 6134A-PRO, and 6132A-PRO each grew progressively in >50% of normal syngeneic C3H/HeN mice when transplanted subcutaneously as solid tumor fragments (Table 1). Based on tumor size, the smallest fragments used contained $\sim 1.5 \times 10^6$ cancer cells (see calculations in Materials and Methods). In contrast, much larger

Table 1. *Tumorigenicity of Cancer Cells Inoculated into Normal Mice as Cell Suspensions or Fragments of Solid Tumors*

Tumor	Inoculum*	Tumor cells	Tumor outgrowth‡
		$\times 10^6$	
1591-PRO	Suspension	50	0/7§
	Suspension	10	1/8
	Fragments	15	11/15
	Fragments	3	10/12
	Fragments	1.5	8/12§
6134A-PRO	Suspension	50	0/5‖
	Suspension	10	0/16
	Fragments	15	9/11
	Fragments	3	8/12
	Fragments	1.5	7/12‖
6132A-PRO	Suspension	50	4/5
	Suspension	10	0/7¶
	Fragments	15	6/8
	Fragments	3	9/10
	Fragments	1.5	7/10¶

* 8–10-wk-old mice were used for tumor challenge. When mice were challenged, the last inoculum was given to a nude mouse to assure the viability of the fragments at the end of the experiment. For each tumor line, 10^6 to 10^7 cells in suspension produced outgrowth of tumors in five of five nude mice.
‡ Number of mice that developed tumors per number of mice challenged. Results involving more than five mice challenged were pooled from two or more experiments, each comparing the outgrowth of a tumor cell suspension with that of solid tumor fragments. Tumor incidence is listed at day 60 after challenge. Mice tumor free after that date were considered cured and the experiment was terminated.
§ The difference between the two ratios with this footnote symbol was found to be significant by the Fisher's exact test ($p < 0.015$).
‖ This difference between the two ratios with this footnote symbol was found to be significant by the Fisher's exact test ($p < 0.05$).
¶ This difference between the two ratios with this footnote symbol was found to be significant by the Fisher's exact test ($p < 0.01$).

doses of these same cells were completely nontumorigenic when administered as suspensions of cells that had been grown in tissue culture. Thus, challenges of 10^7 suspended cells and, for two of the tumors, even 5×10^7 cells (>33-fold more cells than present in the smallest fragments) were rejected by virtually all recipients. These same doses of suspended cells were fully capable of forming tumors in nude mice (data not shown), ruling out the possibility that the particular culture conditions used had rendered the cell suspensions nonviable or otherwise incapable of progressive growth in vivo.

To investigate the reason for this striking difference in tumorigenicity between tumor fragments and cell suspensions, we injected the suspended cells into subcutaneous polyurethane sponge implants. Indeed, the presence of the sponges increased the tumorigenic potential of the cells dramatically (Table 2); the tumor incidence of the 1591-PRO tumor increased from 13 to 90% and from 0 to 100% for the two doses of cells used. Tumor incidence for 6132A-PRO increased from 0 to 67% for the smallest dose of cells while the larger dose produced growing tumors in the absence of the sponges. Little growth occurred for the 6134A-PRO tumor cells in the presence of the sponges, indicating a high degree of immunogenicity that was not overcome.

Table 2. *Some Lines of Cancer Cells in Suspension Are More Tumorigenic after Injection into Subcutaneous Sponges*

Tumor	Tumor cells in suspension	Tumor outgrowth*	
		Sponges	Subcutaneous‡
	$\times 10^6$		
1591-PRO	50	5/5§	(0/7)§
	10	9/10	(1/8)
6134A-PRO	50	1/5‖	(0/5)‖
	10	0/6	(0/16)
6132A-PRO	50	5/5	(4/5)
	10	4/6¶	(0/7)¶

* Number of mice that developed tumors per number of mice challenged. Results involving more than five mice were pooled from two or more experiments, see Table 1. Tumor incidence at day 60 after challenge. Mice tumor free after this time were considered cured and the experiment was terminated.
‡ For comparison, tumor outgrowth of same size inocula injected subcutaneously is shown in parentheses from data presented in Table 1. Sponges were injected in experiments done concurrently with those in Table 1.
§ The difference between the two ratios with this footnote symbol was found to be significant by the Fisher's exact test ($p < 0.002$).
‖ The difference between the two ratios with this footnote symbol was found not to be significant by the Fisher's exact test ($p = 1.00$).
¶ The difference between the two ratios with this footnote symbol was found to be significant by the Fisher's exact test ($p < 0.02$).

Enzymatically digested fragments yielded suspended cells that were rejected by normal mice as effectively as suspended cells obtained from cultures. This was indicated in two experiments showing that all of 10 mice rejected either 5×10^7 or 10^7 cancer cells derived from fragments of 1591-PRO, though these numbers of cells produced growing tumors in five of five nude mice. Thus, stroma appears to be essential for the enhanced growth potential of solid tumor fragments.

Stroma Is also Critical for Determining the Tumorigenicity of Cancer Cells in Tumor-bearing Hosts. Normal tumor-free mice reject regressor tumor fragments at any testable dose, but tumor-bearing animals (TBA) fail to reject regressor tumor fragments (20). TBA fail to reject even small numbers (1.5×10^6) of K^{216} regressor tumor cells embedded in stroma

Table 3. *Stroma Is also Critical for Determining the Tumorigenicity of Cancer Cells in Tumor-bearing Hosts*

Exp.	Host*	Challenge			Take of inoculum/ graft (%)‡
		Type	Cell dose	Stroma	
			$\times 10^6$		
1	Tumor-bearing	K^{216+} tumor suspension	50	None	2/16 (13)
		K^{216+} tumor suspension	10	None	0/8 (0)
		K^{216+} tumor suspension	10	Sponge	5/6 (83)
		K^{216+} tumor fragments	15	C3H/HeN	8/9 (88)
		K^{216+} tumor fragments	5	C3H/HeN	5/5 (100)
		K^{216+} tumor fragments	1.5	C3H/HeN	5/5 (100)
	Tumor-bearing	1591-PRO tumor suspension	50	None	9/9 (100)
		1591-PRO tumor suspension	10	None	8/8 (100)
		1591-PRO tumor fragments	1.5	C3H/HeN	8/8 (100)
2	Tumor-bearing	K^{216+} tumor fragments	15	K^{216+} transgenic	0/20 (0)
		K^{216+} tumor fragments	15	C3H/HeN	16/16 (100)
		K^{216+} skin	–	K^{216+} transgenic	0/6§ (0)
	Tumor-free	K^{216+} tumor fragments	15	C3H/HeN	0/18 (0)
		K^{216+} skin	–	K^{216+} transgenic	0/6§ (0)

* C3H/HeN mice were injected with fragments of the K^{216}-negative progressor tumor 1591-PRO (K^kD^k) at one subcutaneous site (right flank). 4–5 wk later, when tumors had reached an average volume of 2–5 cm^3, these tumor-bearing mice were challenged with the type of tissue indicated (left flanks).

‡ Tumor-free or tumor-bearing C3H/HeN mice (K^kD^k) received K^{216+} transgenic full thickness skin grafts ($K^kD^kK^{216}$) or were injected with one trocar load of the K^{216+} transfected tumor ($K^kD^kK^{216}$) that had been grown up C3H/HeN or K^{216} transgenic mice, or were injected with a suspension of K^{216}-transfected tumor cells. Exps. 1 and 2 were pooled from two or more individual experiments. The last tumor inoculum was given to a nude mouse to assure viability of the fragments until the end of the challenge experiment. Mice that were tumor free at day 60 after challenge were considered cured and the experiment was terminated.

§ Survival of the transplanted K^{216} transgenic skin was 15 (± 1) and 13 (± 1) d (± SD) in tumor-bearing and tumor-free mice, respectively.

but usually do reject as many as 5×10^7 suspended regressor tumor cells (Table 3, Exp. 1). However, just 10^7 suspended regressor tumor cells grew out when injected into sponges in the TBA. The lesser immune resistance of the TBA is also demonstrated by inability to reject suspensions of 1591-PRO cells (compare Table 1 with Table 3, Exp. 1). The results showing higher tumorigenicity of 1591-RE tumor fragments or 1591-RE tumor cells in sponges for TBA (Table 3) are analogous to the findings using 1591-PRO in tumor-free mice (Tables 1 and 2), except that the higher level of immunogenicity of 1591-RE (K^{216} positivity) can compensate for the reduced immune responsiveness of the TBA.

Fragments Consisting of Cancer Cells in Antigenic Stroma Are Less Tumorigenic. TBA reject skin grafts but not malignant tumor grafts even when both the skin and the tumor cells express the same rejection antigen (5). The reason for this difference might be that the rejection antigen in skin is expressed by all cells, whereas the antigen in tumor fragments is expressed only by cancer cells. If this was the reason for the difference in the response of the mice to normal grafts and tumor grafts, then a tumor should be rejected more effectively if the stroma of the tumor also carried the rejection antigen. Indeed, Table 3, Exp. 2 shows that K^{216}-transfected tumors that had grown in K^{216} transgenic mice (K^{216}-positive stroma) were rejected by tumor-bearing mice whereas the same tumor obtained from C3H/HeN nude mice (K^{216}-negative stroma) was not rejected. Nude mice were used because the K^{216}-transfected tumor does not grow in normal mice. In additional experiments, several types of progressor tumor cells were transplanted into normal C3H/HeN or K^{216} transgenic C3H/HeN recipients. The tumors grew similarly, as would be expected since the transgenic mice are genetically identical to C3H/HeN mice except for the additional expression of the K^{216} gene. Solid tumor fragments obtained from these different hosts were then transplanted subcutaneously into normal mice. Transplanted tumors that had grown in the K^{216} transgenic donors and therefore had antigenic K^{216} stroma grew with decreased incidence in normal mice (Table 4) compared with K^{216} transgenic mice. These results indicate that the antigenic stroma can lead to the rejection of cancer cells embedded in it.

Bone Marrow–derived Antigenic Stroma Decreases the Tumorigenicity of Tumor Fragments. The stroma of a graft consists of sessile as well as bone marrow–derived components such as

Table 4. *Antigenic Stroma Makes Cancer Cells Less Tumorigenic*

Host	Fragments*		Tumor outgrowth (%)‡
	1.5×10^7 tumor cells	Stroma	
C3H/HeN	1591-PRO	C3H/HeN	22/31 (71)§
		K^{216+} transgenic	6/38 (16)§
	6134A-PRO	C3H/HeN	9/11 (82)‖
		K^{216+} transgenic	1/12 (8)‖
	6132A-PRO	C3H/HeN	6/8 (75)¶
		K^{216+} transgenic	3/14 (21)¶
K^{216} transgenic	1591-PRO	K^{216+} transgenic	14/18 (78)
		C3H/HeN	12/18 (67)
	6134A-PRO	K^{216+} transgenic	10/12 (83)
	6132A-PRO	K^{216+} transgenic	12/12 (100)

* Tumor-free C3H/HeN (K^kD^k) or K^{216} transgenic C3H/HeN ($K^kD^kK^{216}$) mice were injected with one trocar load of solid tumor fragments of the progressor tumor that had grown for 5 wk or more in C3H/HeN or K^{216} transgenic line as indicated.
‡ Data pooled from two or more experiments. The last tumor inoculum was given to a nude to assure viability of the fragments until the end of the challenge experiment. Mice that were tumor free for 60 d after challenge were considered cured and the experiment was then terminated.
§ The difference between the two ratios with this footnote symbol was found to be significant by the Fisher's exact test ($p < 0.0001$).
‖ The difference between the two ratios with this footnote symbol was found to be significant by the Fisher's exact test ($p < 0.0002$).
¶ The difference between the two ratios with this footnote symbol was found to be significant by the Fisher's exact test ($p < 0.05$).

passenger leukocytes (i.e., leukocytes present in the graft at the time tissue is taken for transplantation). To determine whether these bone marrow–derived components contributed to rejection of tumor grafts, we constructed chimeras in which only the bone marrow–derived cells or the sessile stromal cells expressed the K^{216} antigen. Thus, we reconstituted irradiated K^{216} transgenic mice with C3H/HeN bone marrow or

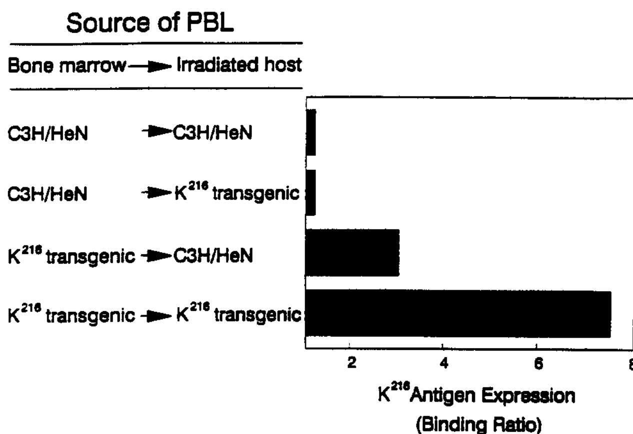

Figure 1. Level of K^{216} antigen expression in peripheral white blood cells of bone marrow chimeras. Bars for each type of chimera represent the level of K^{216} antigen expression above background fluorescence. Results shown here are representative of results obtained in three independent experiments. For details of the generation of chimeras and cytofluorometric analysis, see Materials and Methods.

reconstituted C3H/HeN mice with K^{216} bone marrow from K^{216} transgenic mice. 1591-PRO tumors were grown in each type of chimera to provide a source of tumors containing the different stromal components. Fig. 1 shows that nucleated PBL from chimeric C3H/HeN TBA having been reconstituted with K^{216} transgenic bone marrow showed significant staining with anti-K^{216} antibody; in contrast, PBL of the K^{216} transgenic C3H/HeN chimera reconstituted with C3H/HeN bone marrow showed no K^{216}-positive staining above the background (i.e., the level of fluorescence was equal to PBL from the K^{216}-negative control C3H/HeN TBA). 1591-PRO tumors that had grown in these chimeric hosts were then transplanted into recipients as indicated in Table 5. Transplants of tumors that had grown in syngeneic mice reconstituted with K^{216} transgenic bone marrow were rejected at least as effectively as transplants of tumors that had grown in K^{216} transgenic mice; i.e., the K^{216} bone marrow–derived stromal components were very powerful in decreasing the tumorigenicity of tumor fragments. We cannot assert that K^{216} sessile elements alone can lead to rejection since the irradiated K^{216} transgenic mice with C3H/HeN bone marrow may have had in the graft a few remaining K^{216} bone marrow–derived cells that could not be detected.

Antigenic Tumor Stroma Does Not Decrease the Tumorigenicity of Poorly Immunogenic Cancer Cells. If rejection was caused solely by reaction to stroma, then highly malignant cells in immunogenic stroma should also be rejected. To test this possibility several other progressor tumor cell lines, a sponta-

Table 5. *Bone Marrow–derived Antigenic Stroma Decreases Tumorigenicity of Tumor Fragments*

Exp.	Stroma of tumor fragments*	Tumor outgrowth (%)‡
1	C3H/HeN	8/10 (80)
	K^{216} transgenic	4/13 (31)
	Chimeric: K^{216} transgenic reconstituted with C3H/HeN bone marrow	2/5 (40)
2	C3H/HeN	11/15 (73)§
	K^{216} transgenic	3/15 20)
	Chimeric: C3H/HeN reconstituted with K^{216} transgenic bone marrow	0/10 (0)§
3	C3H/HeN	9/12 (75)‖
	Chimeric: K^{216} transgenic reconstituted with C3H/HeN bone marrow	3/12 (25)
	Chimeric: C3H/HeN reconstituted with K^{216} transgenic bone marrow	1/12 (8)‖
4	C3H/HeN	3/5 (60)
	Chimeric: C3H/HeN reconstituted with C3H/HeN bone marrow	4/5 (80)

* Mice bearing 1591-PRO tumors for at least 4 wk were used; mice received 900 rad of whole body gamma radiation from a ^{137}Cs source. Within 1 h after irradiation, tumor bearers received 1–2 × 10^7 bone marrow cells obtained from the designated source. The irradiated reconstituted tumor bearers were used 6–7 d later as source of solid tumor fragments for tumor challenge. Exp. 4 served as a control showing that irradiation of the tumor donor did not affect the outgrowth of that tumor when transplanted.

‡ Tumor-free C3H/HeN mice were challenged with one trocar load of fragment of 1591-PRO tumors that had grown in regular C3H, chimeric C3H reconstituted with K^{216} transgenic bone marrow, K^{216} transgenic mice, or chimeric K^{216} transgenic mice reconstituted with C3H/HeN bone marrow. Results pooled from two or more experiments. Mice that were tumor free at day 60 after challenge were considered cured and the experiment was terminated.

§ The difference between the two ratios with this footnote symbol was found to be significant by the Fisher's exact test ($p < 0.0002$).

‖ The difference between the two ratios with this footnote symbol was found to be significant by the Fisher's exact test ($p < 0.004$).

neous cancer, a highly malignant UV-induced tumor, and an MCA-induced fibrosarcoma (for which it has been very difficult or impossible to achieve effective immune protection by preimmunization), were prepared in immunogenic stroma. Table 6 shows that as few as 10^4 AG-104A or 1591-MET or 10^6 MC-3152-PRO tumor cells in suspension will usually grow in normal mice. These cancer cells, when grown as solid tumors in normal C3H/HeN or K^{216} transgenic mice and then transplanted into normal tumor-free mice, grew progressively (Table 6). Thus, an immune response to the stroma alone was not sufficient for rejection of poorly antigenic cancer cells.

Discussion

Stroma can be critical for preventing or permitting immunologic destruction of cancer cells in a tumor transplant. Our findings, summarized in Fig. 2, show that suspended cells dispersed in medium and injected subcutaneously are separated from one another, and antigens on the tumor cells may be exposed directly to the host's immune system, which leads to effective immunity. In contrast, cancer cells injected into sponges or injected as tumor fragments are confined together and may fail to induce effective immunity or be protected from destruction by host immunity; however, this protection can be overridden by the presence of antigen on tumor stroma, as indicated by the rejection of cancer cells embedded in transgenic stroma.

We do not know how nonantigenic syngeneic stroma contributes to ineffective immunological rejection of solid tumor fragments; one possibility is that stroma lacking antigenicity or antigen-presenting function may impede migration of tumor-infiltrating lymphocytes, as suggested by in vitro experiments using an artificial collagen matrix (21), or otherwise prevent effective sensitization of immune cells or immune destruction of cancer cells after sensitization has occurred. This notion is consistent with the fact that tumor cells injected into a nonantigenic polyurethane sponge matrix grew out. It is interesting in this regard that allogeneic keratinocytes grafted onto a stroma lacking antigen-presenting cells are not rejected by the host (22). Alternatively, containment of tumor cells in close proximity to one another by the syngeneic stroma may result in crossfeeding by local accumulation of growth-promoting substances produced by the

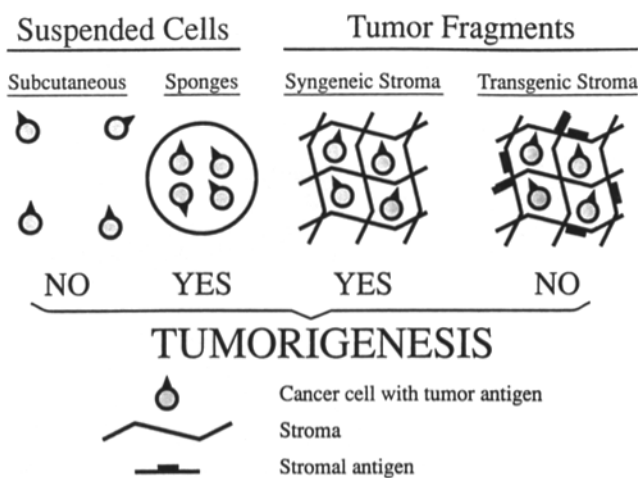

Figure 2. The outcome of inoculated immunogenic cancer cells as a function of stroma.

Table 6. *Antigenic Stroma Does Not Decrease the Tumorigenicity of Poorly Immunogenic Cancer Cells*

Tumor cell line	Challenge*			No. of tumor cells	Outgrowth of tumors‡
	Stroma	Type of inoculum			
AG-104		Suspension		10^7	10/10
				10^4	8/10
	C3H/HeN	Fragments	(1 trocar)	1.5×10^7	13/13
			(0.1 trocar)	1.5×10^6	5/5
	K^{216} transgenic	Fragments	(1 trocar)	1.5×10^7	13/13
			(0.1 trocar)	1.5×10^6	5/5
1591-MET		Suspension		10^7	10/10
				10^4	7/10
	C3H/HeN	Fragments	(1 trocar)	1.5×10^7	10/10
			(0.1 trocar)	1.5×10^6	5/5
	K^{216} transgenic	Fragments	(1 trocar)	1.5×10^7	10/10
			(0.1 trocar)	1.5×10^6	5/5
MC-3152-PRO		Suspension		10^7	9/10
				10^6	4/5
				10^5	1/5
				10^4	0/5
	C3H/HeN	Fragments	(1 trocar)	1.5×10^7	12/12
			(0.1 trocar)	1.5×10^6	3/5
	K^{216} transgenic	Fragments	(1 trocar)	1.5×10^7	12/12
			(0.1 trocar)	1.5×10^6	4/5

* Normal tumor-free C3H/HeN mice were challenged subcutaneously with the inoculum indicated as positive controls, mice were challenged with 1591-PRO fragments in C3H/HeN (4/5) or antigenic K^{216} stroma (0/5) or challenged with 10^7 tumor cells (0/5).
‡ Mice that survived tumor free for 60 d after challenge were considered cured and the experiment was terminated.

tumor cells, a possibility also consistent with the results using sponges. Finally, the interaction between tumor and syngeneic stroma of injected tumor fragments may stimulate angiogenesis or enhance expression of certain integrins or other adhesion molecules that promote earlier or faster tumor growth, and thereby prevent rejection. The poor accessibility of drugs and host defenses to the cancer cells in solid tumors due to stroma could be in some part responsible for the relatively ineffective therapy for solid tumors compared with therapy for hematopoietic malignancies because the cancer cells of solid tumors have a very different relationship to the blood supply (23).

We are also uncertain why antigenic stroma promotes immunological rejection of a tumor. In so far as we can tell, the architecture of the syngeneic and transgenic stroma appears identical, at least initially. Differences in the antigenicity of the stroma appear to be the reason for our previous observation (5) that tumor-bearing mice fail to reject regressor tumors even though these mice can still reject effectively a simultaneous normal graft expressing the same rejection antigen. Our observations that weakly antigenic tumor cells can be rejected when the stroma is antigenic are potentially very important because they indicate that if stromal components of a growing tumor can be rendered antigenic, then the tumor may be rejected as effectively as an allogeneic skin graft. Although antigenic stroma is a laboratory artifact in the present studies, some tumors may induce autogenous stromal antigens (2), so possibly, such antigens could be induced in the stroma in other tumors by therapy. Our finding that bone marrow–derived components of antigenic stroma, presumably comparable with passenger leukocytes in allografts, are alone sufficient to cause tumor rejection of immunogenic tumors suggests the possibility of targeting such immune therapy to circulating leukocytes that specifically localize to the tumor. Such leukocytes obtained from tumor-specific cytolytic T cell clones and made highly antigenic to the host by transfection with a gene encoding an allogeneic MHC class I antigen, for example, may localize in the tumor and act as inducer and target of a second newly induced potent antiallogeneic immune response. Thus, understanding the basis for the enhanced rejection of cancer cells within an antigenic matrix may help us to develop new strategies for promoting immunologic rejection of established solid tumors.

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