Accelerated Growth of Melanomas after Specific Immune Destruction of Tumor Stroma in a Mouse Model

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Abstract

 Destruction of the entire stroma in a tumor could provide a stringent test of the prospects for tumor eradication in a single treatment. This possibility was investigated by experimental immune destruction of the stroma in a mouse melanoma model. Melanomas were first produced by grafting skin from transgenic C57BL/6 females of high-melanoma susceptibility to low-susceptibility transgenic males so that the malignant cells would be genetically female and the stromal cells genetically male. Subcutaneous transplant lines were then established from the melanotic and the amelanotic zones of such a melanoma and were carried in transgenic male hosts to ensure the male composition of the stroma. Thus, the male-specific H-Y histocompatibility antigen, which is ubiquitously expressed on male cells, could provide the target for an immune attack against the stroma. The transplant lines were next passaged once in transgenic females preimmunized against the H-Y antigen by having received and rejected a graft of C57BL/6 nontransgenic male skin. The antistromal immune response of these hosts did not prevent recovery of the tumors, which required a substantially prolonged latency. However, after retransplantation to nonimmunized males and females, the latency was markedly shortened from the original level. Thus, the treatment had indirectly selected for more rapidly growing tumor cells and hastened malignant progression.

Introduction

Tumors, like normal organs, are composed of stromal, as well as parenchymal, elements. Generally included in the stroma are the endothelial, smooth muscle, and hematopoietic cells of the vasculature; fibroblasts and other connective tissue cells; and extracellular products found in basement membranes, in collagen and other fibers, and in the extracellular matrix. The importance of the vascular component stems from the limits of diffusion: as the tumor size approaches a few mm in diameter, new vascular sprouts are required (1). The other stromal constituents may play many vital roles in the tumor economy. These involve receptor-mediated signal transduction pathways and other mechanisms capable of influencing cell anchorage, migration, and mitotic activity. Thus, interactions with the stroma contribute to tumor growth, progression, and dissemination (2–10). New antitumor strategies aimed at destroying one or another member of the stromal support system are in fact being increasingly explored as alternatives or supplements to agents injurious to the malignant cells themselves.

The most draconian treatment of this sort would be the specific obliteration of the entire tumor stroma. Although not now clinically feasible, its therapeutic potential and its possible consequences can be tested in a laboratory model involving transgenic mouse melanomas. In Tyr-SV40E transgenic mice, the transgene is specifically transcribed in pigment cells because of a 5′ controlling segment of the mouse tyrosinase gene (11). The SV40 oncogenic sequence apparently acts as an initiator; to obtain malignant skin melanomas, the melanocytes must be exposed to a promoting stimulus [e.g., to UV radiation (12, 13) or to conditions associated with wound healing (14)]. Although the mice all have the same inbred-strain genetic background (C57BL/6), there are separate lines, each descended from a single egg injected with transgene DNA and distinguished by a particular level of transgene expression and, correspondingly, of melanoma inducibility. When skin is grafted from donors of a line with high expression (e.g., line 8) to hosts of a low-expressing line (e.g., line 12), melanomas reliably arise in the grafts and metastasize into the organs of the hosts. Some of the melanomas are zonal tumors in which distinct melanotic and relatively amelanotic parts are present. As in their human counterparts, the amelanotic component has more mitotically active cells and is comparatively more advanced in malignancy.

Female-to-male grafts are accepted in syngeneic mice, whereas male-to-female grafts have long been known to be rejected (15–17). Rejection is attributable to Y-linked sequences (18) ubiquitously expressed in genetically male cells and responsible for the H-Y histocompatibility antigen. The antigen has recently been identified as a peptide product originating from the Smcy gene in the mouse (19); its homologue has been found in the human genome (20). We have generated mouse melanomas for the present study by grafting female transgenic skin to transgenic males so that the host-derived stroma could later be selectively eradicated by transferring the tumor to females preimmunized against the H-Y antigen.

Materials and Methods

Origin of Melanomas. To obtain female melanomas with male stroma, skin was grafted from C57BL/6 transgenic hemizygous females of line 8 to C57BL/6 transgenic hemizygous males of line 12. Mice of the donor line are characterized by high melanoma susceptibility but have a short life span because of early eye melanomas; mice of the host line have low melanoma susceptibility and a relatively long life span (11). The graft arrangement thus allows the skin to be exposed to wound-healing conditions and to inhabit a long-lived host. A 1-cm disc of full-thickness skin from the dorsal trunk was grafted according to standard procedures (14). The graft was scraped free of underlying fat and placed on the lateral trunk of an anesthetized host from which a slightly larger piece of skin was removed. Bandages were left in place for 9 days. In such grafts, the malignant cells and their metastases originate from the grafts (14).

After advanced melanomas had developed in the grafts (Fig. 1, experiment 1), one (case 155) was selected for further work. This was one of several zonal melanomas in which a darkly melanotic zone and a larger, relatively amelanotic zone were clearly distinguishable. As in our other zonal melanomas, these exemplified different stages in malignant progression, of which the amelanotic zone had a larger number of cells in mitosis4 and was the more

4 Unpublished data.
### Results and Discussion

During serial transfer in males, the latency interval between inoculation and external detection of a tumor became stable in each line after the first transplant generation. This averaged 38 days for the melanotic and 15 days for the amelanotic line; the latter period was 60% shorter, as recorded in Fig. 2 for the transplant generations marking the start of the experiment (groups IA and IIA). Faster growth was consistent with the larger size of the amelanotic zone in the original case 155 tumor.

Transfer to immunized females prolonged the latency for each transplant line (Fig. 1, experiment 4). Although the average absolute increase was greater for the melanotic line, the percentage increase was virtually identical in both: 21% for the melanotic line (Fig. 2, group IB) and 20% for the amelanotic line (group IIB). The intensity of the immune response of females to the H-Y antigen in syngeneic male grafts is determined largely by immune-response genes associated with the MHC (17). Females from strains of the H@2b haplotype, including C57BL/6, not only reject male skin grafts from the same strain, but subsequently reject a second male graft more rapidly than the first. In the immunization step of the study (Fig. 1, experiment 3), transgenic females rejected the first male grafts (skin) within an advanced. The presence in mouse 155 of both melanotic and amelanotic lymph node metastases (largely the latter) attested to the malignancy of the primary tumor. Tumor was removed from each zone of the skin melanoma, and 8–10 fragments approximately 1 mm³ in size were transplanted into line 12 hemizygous males s.c. The elapsed time for external detection of a tumor was recorded for each host.

#### Immunization against the Male Antigen

Transgenic female hemizygotes of line 12 were immunized against the male-specific H-Y antigen by applying a C57BL/6 nontransgenic male skin graft (Fig. 1, experiment 3). After the male grafts had been rejected, these females served as recipients for s.c. tumor transplants from the respective melanotic and amelanotic transplant lines (Fig. 1, experiment 4).

### Table: Aim and Experiment

<table>
<thead>
<tr>
<th>Aim</th>
<th>Experiment</th>
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<tbody>
<tr>
<td>1. Induction of skin melanomas to be comprised of ♀ tumor cells and ♂ stromal cells</td>
<td><img src="image1.png" alt="Diagram" /></td>
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<tr>
<td>2. Establishment of a melanotic and an amelanotic transplant line from a ♀/♂ zonal melanoma by serial growth in ♂♂</td>
<td><img src="image2.png" alt="Diagram" /></td>
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<tr>
<td>3. Immunization of ♀♀ against ♂ antigen</td>
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<td>4. Elimination of stromal (♂) cells in one generation of each transplant line</td>
<td><img src="image4.png" alt="Diagram" /></td>
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<td>5. Test of selection for more rapidly growing tumor cells in the melanotic line</td>
<td><img src="image5.png" alt="Diagram" /></td>
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ACCELERATED MELANOMA GROWTH AFTER STROMA DESTRUCTION

Fig. 2. Scatter plot of tumor latencies after inoculation of transgenic hosts with the melanotic (groups IA–IC) or amelanotic (groups IIA–IIB) transplant lines derived from the same zonal melanoma. Points, time in days between s.c. inoculation of small tumor fragments and macroscopic detection of tumor in a single host. Groups IA and IIA are represented as experiment 2 in Fig. 1, groups IB and IIB correspond to experiment 4, and group IC corresponds to experiment 5.

<table>
<thead>
<tr>
<th>Hosts</th>
<th>αα</th>
<th>αα + αα+</th>
<th>αα</th>
<th>αα</th>
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<tr>
<td>n</td>
<td>13</td>
<td>20</td>
<td>13</td>
<td>12</td>
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<tr>
<td>Average latency</td>
<td>36 days</td>
<td>46 days (21%†)</td>
<td>23 days (39%‡)</td>
<td>15 days</td>
</tr>
<tr>
<td>Group</td>
<td>IA</td>
<td>IB</td>
<td>IC</td>
<td>IIA</td>
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average of 40 days (range, 28–56 days). Although the elapsed time for rejection of the second male grafts (tumor stroma) cannot be directly ascertained, the observed latencies in tumor growth suggest that an accelerated immune response was indeed mounted against the male tumor stroma as compared with the prior response against male skin. This is supported by the fact that the average latencies after tumor inoculation (46 days for melanotic grafts in Fig. 2, group IB, and 18 days for amelanotic grafts in Fig. 2, group IIB) must encompass not only male stromal destruction but also stromal replacement from the hosts, neovascularization, and substantial growth before the tumor is detectable. Upon dissection, the tumors appeared well vascularized and resembled those of their respective earlier transplant generations.

To learn whether recovery from the destruction of stromal cells had affected the accompanying population of malignant cells, a melanotic tumor appearing after a 40-day latency in one of the immunized females was retransplanted to nonimmunized females and males (Fig. 1, experiment 5). (The amelanotic tumor was not retransplanted because data for very short latencies are less reliable.) The results for the melanotic tumor were unexpected and striking: in hosts of each sex, the latency was decreased to an average of 23 days (Fig. 2, group IC). In comparison with the unperturbed melanotic tumor before anti-H-Y treatment (group IA), this was a 39% decrease on average. The shortest individual latencies in the melanotic line (group IC) now overlapped with the longest ones in the amelanotic line (group IIA). That this was not a transient acceleration was documented by maintenance of approximately the same level in the three subsequent transplant generations: in a total of 25 nonimmunized hosts, an average latency of 24 days was obtained (data not shown). It is noteworthy that at the end the tumors were still darkly pigmented, thereby suggesting that subpopulations of tumor cells differing in growth potential but still relatively well differentiated as pigment cells were already present in the melanotic line. Presumably the temporary loss of stroma and disruption of tumor organization, and perhaps a wound-healing process (14) resulting from this loss, have indirectly favored the upsurge of more rapidly growing malignant cells that were previously in a small minority or quiescent.

The P values were calculated with the Mann-Whitney U test (21) for paired comparisons documented in Fig. 2. These comparisons were between group IA and groups IB, IC, and IIA and between group IIA and group IIB. All of the P values were clearly significant, ranging from <.01 to <.0001.

Thus, an acute exposure to antistromal treatment delayed, but did not prevent, growth of the melanomas. However, the status quo was not restored after treatment; instead, malignant progression was in fact accelerated. Would repetitions of a comparable immune strategy continue to select for even more malignant cells? The question is academic insofar...
as even a single treatment capable of specifically eliminating all the stroma in a human tumor may not soon be achievable in clinical practice. A more relevant question might be whether a therapy more limited in scope (i.e., aimed at only one stromal component) and necessitating chronic long-term treatment might gradually impose enough selective pressure to favor the more proliferative tumor cell variants during intermittent periods of tumor recovery.

References

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