Growth-regulatory Control of Human Cell Hybrids in Nude Mice

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ABSTRACT

The role of natural killer (NK) cells in the control of growth of human cell hybrids in nude mice was evaluated. Both nontumorigenic and tumorigenic HeLa-fibroblast hybrids were highly sensitive to NK-mediated cytotoxicity, but neither hybrid induced such activity when injected into nude mice. Furthermore, tumorigenic hybrids grew in mice which had high levels of NK activity induced by i.p. inoculation of Corynebacterium parvum vaccine. Histological examination of the nontumorigenic and tumorigenic populations inoculated s.c. into nude mice indicated that both populations initially divided actively for the first 3 to 4 days. After this time, the nontumorigenic cells showed a dramatic decline in mitotic activity accompanied by a morphological shift to a more fibroblastoid appearance. The cells remained in the animal as a viable nondividing tissue. The tumorigenic population continued to actively divide and produced a large progressively growing tumor. This series of events determined from histological examination was supported by kinetic studies. These results suggest that NK cells play no role in the suppression of growth of the nontumorigenic hybrid cells and that host-mediated growth-regulatory control is responsible for the shutdown of mitotic activity of these cells without causing their death.

INTRODUCTION

The transformation of a normal cell into a neoplastic one involves a multistep progression which is probably under multigenic control (1, 4, 11). Analysis of the events leading to the neoplastic state has been facilitated by the use of cell hybrids derived from the fusion of normal and malignant parental cells. In many cases, tumor-forming ability is suppressed in such hybrids, although the cells continue to express many traits characteristic of transformed cells in vitro (14, 33, 36). By using somatic cell fusion coupled with techniques such as transfer of metaphase chromosomes or naked DNA (23, 38), it may well be possible to identify those genetic elements responsible for the control of the malignant and transformed phenotypes in cultured cells.

An important caveat, however, should be considered when interpreting assays of the tumor-forming ability of mammalian cell populations. Since tumorigenicity can be assayed only in animals and the cells under test are often allogeneic or xenogeneic, immunodeficient animals must be used. Erroneous interpretations regarding the tumor-forming ability of cells have occurred due to inadequate immunosuppressive procedures (17). In recent years, the congenitally athymic nude mouse has become a popular experimental host for tumorigenicity studies (13, 27, 31, 35). However, the use of this animal as an adequate model for tumorigenicity testing has been questioned. Although the nude mouse lacks thymus-dependent or T-cell-mediated immune function, it possesses high levels of natural cell-mediated cytotoxicity or NK activity (15, 18). This NK activity has been shown to inhibit the growth of certain tumorigenic cells in nude mice (19, 37).

We have been studying the genetic control of cancer and transformation using human cells and mouse-derived human cell hybrids exclusively and have found that the hybrid cells derived from malignant x normal cell fusions do not form tumors in nude mice (30, 33). Rare tumorigenic segregants arise from these suppressed hybrids which do form progressive tumors in nude mice. The studies reported here use these hybrid cell lines to measure the effect that NK cell activity has upon their tumor-forming ability and also provide formal evidence for growth-regulatory control in vivo.

MATERIALS AND METHODS

Cell Lines. The human cell lines used in this study were a series of nontumorigenic HeLa-fibroblast cell hybrids and tumorigenic segregants derived from them. The origins of these cell lines are summarized in Table 1 and described in greater detail elsewhere (8, 30). The mouse mastocytoma line P815 was selected for comparative purposes in the NK cell cytotoxicity assays (16). All cell lines were maintained in culture using Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, hereafter referred to as growth medium.

Mice. Specific-pathogen-free congenitally athymic BALB/c nu mice (on the BALB/cAnBradleyWehi background) were supplied from stocks bred in this institute. Unless stated otherwise, the mice were derived from matings between nu/nu BALB/c females and BALB/c nu males.

Effector Cells. Cytotoxic effector cells were obtained from peritoneal exudates, spleen, and lymph nodes of 5- to 8-week-old BALB/c nu mice. Cells were obtained from untreated mice, tumor-bearing mice, or animals in which NK activity had been stimulated by i.p. injections of 50 µg of inactivated Corynebacterium parvum vaccine (Coparvax; Wellcome Foundation, London, England; Batch BA3968, 7 mg dry-weight organisms per ml) 4 days prior to harvest of the effector cells (22).

Separation of Adherent and Nonadherent Cells. Removal of adherent cells by nylon wool columns was accomplished using a modification of methods described previously (29). Briefly, 5 to 10 x 10⁶ cells were pipetted onto the top of a nylon wool column packed into a 10-ml plastic syringe. The column had been preswathed and saturated with growth medium. The cells were allowed to enter the nylon wool matrices by gravity elution and were then incubated at 37°C for 45 min.

1 The abbreviations used are: NK, natural killer; C. parvum, Corynebacterium parvum; i.e., intracerebral; HGPRT, hypoxanthine guanine phosphoribosyl transferase; PEC, peritoneal exudate cells.

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The nonadherent cells were then eluted with 30 ml of prewarmed growth medium. The majority of NK cell activity resided in this fraction. Adherent cells were removed from the columns by elution with at least 30 ml ice-cold growth medium coupled with gentle squeezing of the nylon wool column. This cell population consists, in part, of B-cells, macrophages, and cells which can mediate antibody-dependent cytotoxicity (21).

Cytotoxicity Assay. Effector cell cytotoxicity directed against 51Cr-labeled target cells was measured in a 4-hr chromium release assay by a minor modification of the method described by Ceredig (7). A 4-hr assay was used in order to ensure measurement of NK activity and avoid complications such as macrophage-mediated killing, which may occur with a longer incubation period. Maximum release was measured by lysing 1 × 10⁴ target cells with detergent. Spontaneous release was <10% of the maximum release counts. The percentage specific release of 51Cr in the individual wells was calculated as:

\[
\text{Experimental cpm} - \text{spontaneous cpm} \\
\text{Maximum cpm} - \text{spontaneous cpm} \times 100
\]

The percentage of specific release in each case is expressed as the mean of triplicate samples. The standard error of the mean rarely exceeded 2%.

Tumorigenicity Assays. The human hybrid cell lines were grown as monolayer cultures. Confluent cultures of cells were harvested with EDTA and suspended in growth medium. Unless otherwise stated, 1 × 10⁷ cells were inoculated into each of at least 3 mice for each assay. Weanling mice were inoculated s.c., i.m., i.p., and i.c. Newborn mice derived from homozygous nu x nu crosses were inoculated s.c. In the irradiation experiments, 5-week-old mice received 5/740 rads from a Philips RT-250 X-ray machine several hr prior to the inoculation of cells s.c.

Histopathology. Tumors were excised, fixed in 10% buffered formalin, and processed according to conventional techniques (26). Sections were routinely stained with hematoxylin and eosin.

Table 1

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>ESH5</td>
<td>Nontumorigenic HeLa-fibroblast hybrid</td>
</tr>
<tr>
<td>ESH5T</td>
<td>Tumorigenic segregant of ESH5</td>
</tr>
<tr>
<td>ESH5TR</td>
<td>Cell population reestablished in culture from an ESH5T tumor in a nude mouse</td>
</tr>
<tr>
<td>ESH39</td>
<td>Nontumorigenic HeLa-fibroblast hybrid</td>
</tr>
<tr>
<td>ESH39T</td>
<td>Tumorigenic segregant of ESH39</td>
</tr>
<tr>
<td>ESH15T</td>
<td>Tumorigenic HeLa-fibroblast hybrid</td>
</tr>
<tr>
<td>P815</td>
<td>Mouse mastocytoma cell line</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Cell line</th>
<th>No. of cells inoculated</th>
<th>Newborn</th>
<th>Weaning</th>
<th>Irradiated</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESH5</td>
<td>2 × 10⁷</td>
<td>0/4</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>ESH5T</td>
<td>1 × 10⁴</td>
<td>3/3</td>
<td>2/2</td>
<td>3/3</td>
</tr>
<tr>
<td>ESH39</td>
<td>1 × 10⁷</td>
<td>0/4</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>ESH39T</td>
<td>5 × 10⁵</td>
<td>2/2</td>
<td>2/2</td>
<td>3/4</td>
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Kinetic Analysis of Cell Proliferation in Nude Mice. The growth behavior of mixed populations of nontumorigenic and tumorigenic hybrid cells in nude mice was assayed using genetic markers specific for each cell type. The nontumorigenic hybrid ESH5 clone B3, which is HGPRT sensitive and ouabain resistant, was mixed in varying ratios with a tumorigenic segregant, ESH15T, which is HGPRT positive and ouabain resistant. Mixtures of cells were prepared by dispersion of cell monolayers with EDTA and resuspension of the cell populations in growth medium. ESH5 clone B3:ESH15T ratios of 9:1 and 2:1 were tested in these experiments. The cell mixtures were injected s.c. into the ventral midline of each mouse. Approximately 5 × 10⁶ cells were injected into each site. At daily intervals following inoculation, duplicate nodules were excised and gently minced in growth medium. The cell mince was allowed to stand in a tube for 2 min to allow large aggregates to settle out. The remaining cell suspension was distributed in equal volumes into 100-nm Petri dishes containing growth medium alone, which served as the control, and growth medium containing hypoxanthine-aminopterin-thymidine or 6-thioguanine (15 μg/ml). The latter 2 media selected for HGPRT-positive and HGPRT-negative cells, respectively. The dishes were incubated at 37°C for periods up to 10 days, with one medium change, and colonies were then counted after fixation with methanol and staining with Giemsa. The few contaminating mouse fibroblast colonies were readily distinguishable by morphology.

RESULTS

Tumorigenicity Assays. In agreement with previous studies (33), large numbers of nontumorigenic hybrid cells inoculated into various tissues of nude mice failed to form tumors (Table 2). In contrast, tumorigenic segregants formed tumors in virtually 100% of the animals. In this report, the tumorigenicity assays were extended to include newborn nude mice, newborns derived from homozygous nu x nu x nu crosses, and heavily irradiated weanling nude mice. These additional regimens were examined because recent reports have indicated that these animals support the growth of certain virally transformed cells, whereas adult nude mice do not (12, 24). As seen in Table 2, the cell lines ESH5 and ESH39 did not form tumors under these conditions, whereas tumorigenic segregants derived from them did.

NK Cell Activity. The sensitivity of the human hybrid cells to NK cell cytotoxicity was measured in a 51Cr release assay using a 4-hr incubation period. Spleens, inguinal lymph nodes, and PEC derived from unprimed and C. parvum-primed mice...
were used. No cytotoxicity directed against the hybrid cells was found in the unprimed mice (Table 3). Significant killing was obtained when NK activity was induced by C. parvum. Both nontumorigenic and tumorigenic hybrid cells were equally susceptible to killing. The highest killing activity resided in the nonadherent population, thereby strengthening the contention that we were measuring NK activity. Compared to P815, the human hybrid cells were very sensitive to the NK-mediated cytotoxic activity. The enhancement of NK activity by injections of C. parvum has been reported previously (25).

In order to establish whether the nontumorigenic or tumorigenic hybrids induced NK activity, 5- to 7-week-old nude mice were given s.c. injections of $1 \times 10^7$ ESH5 or ESH5T cells. PEC, spleens, and lymph nodes were harvested at Days 4 and 12 following cell injections. These times were selected because Day 4 represents the time when significant NK activity is present following C. parvum priming and because, by Day 12, the nontumorigenic ESH5 nodule is no longer palpable. As illustrated in Table 4, neither cell population induced NK activity in nude mice. NK activity was also not induced by i.p. inoculation of hybrid cells (data not shown). The salient features of the above data are summarized in Chart 1. In addition (Chart 1D), the tumorigenic hybrid ESH5T was injected s.c. into mice which had been primed 4 days previously with an i.p. injection of 50 $\mu$g C. parvum vaccine. On Day 5 following the injection of tumor cells, a second 50-fig dose of C. parvum vaccine was given; on Day 10, the animals were sacrificed and the PEC were harvested. At the time of sacrifice, the animals were bearing tumors at least 1.0 cm in diameter. Histological examination of the tumors showed no evidence of rejection. In the NK cytotoxicity assay, significant killing activity (Chart 1D) was found against both nontumorigenic and tumorigenic hybrids, including the tumor reconstituted cell line ESHST. Thus, tumorigenic hybrids are able to grow and form tumors even in the presence of high NK activity, and those cells which do form tumors are no more resistant to NK cytotoxicity than are those that do not form tumors.

**Histological Aspects of in Vivo Growth Behavior.** Examination of tissue sections of s.c. nodules of the nontumorigenic hybrid ESH5 and its tumorigenic segregant ESH5T during the first few days after injection revealed remarkable similarities in their growth behavior. Within 1 day after injection, the majority of the cells in the center of the tumor nodules were dead (Figs. 1A and 2A). However, in both cases, the periphery of the nodule was composed of healthy viable cells, and mitotic activity was apparent (Figs. 1B and 2B). The residual healthy cells of both ESH5 and ESH5T continued to divide for a further 3 to 4 days. During this time, no differences in morphology or mitotic activity were discernible between the 2 populations (Figs. 1C and 2C). During this period, the dead cells in the center of the nodules disappeared, and the spaces began to fill with healthy cells. By Day 4 and 5, a distinct decrease in the mitotic activity of the nontumorigenic ESH5 cells and reorientation of the cells into a more fibroblastoid appearance was observed (Fig. 1D). The tumorigenic ESHST cells did not undergo this morphological change, and there was no decrease in mitotic activity (Fig. 2D). By Day 7, all mitotic activity had

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**Table 3**

| NK cell activity against human hybrid cells in unprimed and primed nude mice |
|-----------------------------|------------------|------------------|------------------|------------------|
|                              | ESH5  | ESH5T | ESH59 | ESH15 | P815 |
| Effectors                   |       |       |       |       |      |
| Untreated mice              |       |       |       |       |      |
| Spleen                      | <1    | <1    | <1    | <1    | 2    |
| Lymph node                  | <1    | <1    | <1    | <1    | <1   |
| PEC                         | <1    | <1    | <1    | <1    | <1   |
| C. parvum-treated mice      |       |       |       |       |      |
| Spleen                      | 28    | 20    | 20    | —     | 10   |
| Lymph node                  | 15    | 19    | —     | —     | —    |
| PEC                         | 68    | 76    | 65    | 72    | 38   |
| Unfractionated              | 49    | 60    | 52    | —     | —    |
| Nonadherent                 | 81    | 85    | 79    | —     | —    |

* — no assay performed.

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**Table 4**

<table>
<thead>
<tr>
<th>NK cell activity in tumor-bearing nude mice</th>
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<tr>
<td>Nude mouse treatment</td>
</tr>
<tr>
<td>1. PEC harvested 4 days after mice were inoculated with $1 \times 10^7$ ESH5 cells s.c.</td>
</tr>
<tr>
<td>2. Same treatment as Line 1. PEC were harvested at Day 12.</td>
</tr>
<tr>
<td>3. PEC harvested 4 days after mice were inoculated with $1 \times 10^7$ ESH5T cells s.c.</td>
</tr>
<tr>
<td>4. Same treatment as Line 3. PEC were harvested by Day 12.</td>
</tr>
</tbody>
</table>

* Splenens and lymph nodes isolated from the same animals also contained no cytotoxic activity (data not included).
ceased in the ESH5 nodule, and the fibroblastoid shift in morphology had become very pronounced (Fig. 1E), giving the appearance of a nondividing tissue graft. In contrast, the ESH5T cells continued to actively divide (Fig. 2E) and went on to produce large progressive tumors. It should be noted that the nodules of both cell types were well vascularized at this stage and that there was no significant lymphoid cell infiltration.

Kinetic Aspects of In Vivo Growth Behavior. When nontumorigenic and tumorigenic hybrid cells in various ratios were injected as a mixture s.c. into nude mice, virtually pure populations of genetically marked tumorigenic ESH15T cells were recovered by Day 10. The results of an experiment using a 9:1 nontumorigenic:tumorigenic mixture are presented in Chart 2. The relative proportion of HGPRT-positive nontumorigenic ESH5 cells compared to that of the HGPRT-negative tumorigenic ESH15T cells in the tumor nodules remained constant for 3 days. Thereafter, a dramatic decline in the proportion of ESH5 cells was found, coupled with a corresponding increase in ESH15T cells. By Day 7, virtually 100% of the cells recovered from the tumor nodules were tumorigenic ESH15T cells.

DISCUSSION

For the past few years, we have been developing an intraspecific human cell hybrid system whereby we are able to distinguish the separate genetic control of the tumorigenic and transformed phenotypes (30, 33). When normal human fibroblasts are fused with malignant HeLa cells, hybrids are formed which are nontumorigenic in nude mice. Rare tumorigenic segregants spontaneously arise, and these segregants are chromosomally and biochemically very similar to their nontumorigenic hybrid counterparts (8, 30).

In any analysis of the neoplastic nature of cells, it is imperative that the animal assay system for determination of tumorigenicity be adequate. The congenitally athymic nude mouse has been very useful for such studies since it will accept and maintain many tissues, both normal and neoplastic, including many human tumors (13, 27, 31). However, the use of nude mice as suitable animals for tumorigenicity assays has recently been questioned. Several lymphoid cell lines produce tumors when injected i.c. but not when injected s.c. (2, 9, 32). Certain virus-transformed cells will not form tumors in adult nude mice but will do so in newborn animals (12). Finally, tumorigenic cells persistently infected with viruses and also a number of neoplastic lymphoid cells will not produce tumors in nude mice unless the animals have been heavily irradiated (24).

In this report, we show that the HeLa-fibroblast hybrid cells which do not form s.c. tumors in nude mice also do not form tumors when injected i.c. or into newborn or heavily irradiated nude mice, even when more than 1 x 10^7 cells are injected. Tumorigenic segregants derived from these suppressed hybrids, on the other hand, readily form tumors under these conditions when as few as 5 x 10^5 cells are injected.

The critical question remains whether the nontumorigenic hybrid cells are indeed suppressed for their tumorigenic phenotype or whether nude mice actually reject these cells by some immune mechanism, such as natural cell-mediated immunity. There is a considerable body of literature (15, 18, 19, 37) which implicates NK activity in the resistance of nude mice to a variety of tumors. It has been shown, for example, that NK-sensitive tumor cells will not form tumors in mice with high NK activity, whereas NK-resistant cells will (19, 37). It is not known what target antigens NK activity is directed against, but it has been shown that mouse NK cells are cytotoxic for many normal and transformed cell lines, including those of human origin. It is clear in the studies reported here, however, that NK activity plays no role in the inability of the non-tumorigenic HeLa-fibroblast hybrids to form tumors in nude mice. Both the nontumorigenic and tumorigenic hybrid cell populations are very sensitive to NK-mediated cytolysis in vitro. However, neither cell population induced NK activity in nude mice. Furthermore, tumor-derived hybrid cells reestablished in culture were equally sensitive to NK cytolysis; thus, NK-resistant cells are not selected for during tumor growth. Another key finding was that NK-sensitive tumorigenic hybrids grew and formed tumors in nude mice which had been treated with C. parvum vaccine to produce high levels of NK activity. Although high levels of NK cell activity did not prevent the formation of tumors when large numbers of tumor cells were injected, it is of course possible that an effect may be found when lower numbers of tumor cells are used. The further possibility that NK cells are important in the monitoring and eradication of spontaneously occurring nascent tumors remains to be elucidated.

Having established that NK plays no role in the suppression of growth of the nontumorigenic HeLa-fibroblast hybrids, the intriguing question is what is happening to these cells. The histological studies reveal some clues as to why the nontumorigenic cells fail to form tumors in nude mice. One striking feature, which is probably unrelated to the overall growth
control phenomenon, was the rapid death of the majority of the cells injected, no matter whether they were tumorigenic or not. The reason for this is unknown, but the finding should stimulate inquiry as to the relevance of determinations of viable cell inoculum for 50% tumor takes when cells are injected in large numbers s.c., a site which is commonly used for such measurements.

Both the nontumorigenic ESH5 and tumorigenic segregant ESHST populations were indistinguishable in their proliferative behavior and morphology in the mouse for the first 3 to 4 days. During this period, the host is presumably responding to the presence of alien dividing cells by releasing growth-regulatory signals. By Day 4, the nontumorigenic cells receive and respond to these signals by terminating their mitotic activity. At the same time, they also undergo a morphological alteration to a more fibroblastoid shape. Histological examination showed that, at this stage (Days 4 to 6), the nontumorigenic cell nodules were highly vascularized. Thus, angiogenesis factor, which causes tumor vascularization and allows tumor growth (10), is not limiting here. Furthermore, no lymphoid cell infiltration was seen, confirming our supposition that the nontumorigenic cells were not being rejected via some immune mechanism. The nondividing ESH5 cells remained in the animals for some time as a dormant tissue. In other studies (data not reported here), we have obtained viable cells from this tissue and reestablished them in culture up to 15 days after injection into the animal. A similar form of growth control was previously noted by Stiles et al. (34).

In contrast to the above sequence of events with the nontumorigenic hybrid cells, the tumorigenic cells in the same animal either did not provoke the putative host-mediated growth regulatory signal or did not receive or respond to it, thereby evading the host-mediated growth control mechanism. These cells continued to proliferate and formed large progressive tumors.

The hypothetical host-mediated growth control described above is supported by kinetic analysis, in which mixtures of genetically marked tumorigenic and nontumorigenic cells were injected into the same site. Following a period of 3 to 4 days, during which time the ratio of nontumorigenic to tumorigenic cells was maintained, the nontumorigenic cells rapidly declined and were replaced by a virtually pure population of tumorigenic cells. In agreement with the histological observations, this result strongly suggests that both populations initially proliferated at the same rate. By Day 4, the nontumorigenic population had ceased to divide and was diluted out by the rapidly increasing number of tumorigenic cells. This result also establishes another important point, that mixtures of nontumorigenic and tumorigenic cells have no influence upon each other's growth in the animal.

The proposed host-mediated growth-regulatory control described in this study is somewhat analogous to the concept of chalone (5, 28). However, in this case, we envisage the host tissue responding to foreign dividing cells. That is, we postulate that the nontumorigenic HeLa-fibroblast hybrid cells are prevented from proliferating in an uncontrolled fashion by the intervention of host-mediated factors which effectively inhibit mitotic activity without destroying the cells which come under this growth-inhibiting influence. One noteworthy aspect of these studies is that the nontumorigenic HeLa-fibroblast hybrids are chromosomally and biochemically very similar to the tumorigenic segregants derived from them. In particular, both hybrid types have virtually identical growth kinetics in vitro, they have the same decreased requirement for serum growth factors, and both are anchorage independent (33). Thus, although there is currently much interest in growth factors necessary for the proliferation of normal versus transformed cells in vitro (3, 6, 20), perhaps the crucial level of growth control which determines the neoplastic nature of a cell is that which operates in the intact animal and which is separate and distinct from those currently measured under in vitro culture conditions.

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REFERENCES

22. Macfarlane, R. I., Ceredig, R., and White, D. O. Comparison of natural killer

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Fig. 1. Histological appearance of nontumorigenic HeLa-fibroblast hybrid ESH5 injected s.c. into nude mice. A, 24-hr nodule. Note the central necrosis where most of the cells are dead. The periphery of the nodule is composed of healthy cells. H & E, × 100. B, higher magnification of A showing the healthy peripheral cells. Arrows, dividing cells. H & E, × 250. C, 3-day nodule. The cells retain their healthy appearance, and many mitotic cells are evident (arrows). H & E, × 250. D, 5-day nodule. Mitotic activity has virtually ceased. The cells are beginning to elongate into a more fibroblastoid shape. H & E, × 250. E, 7-day nodule. Fibroblastoid morphology is now very distinct. There is a total cessation of mitotic activity. Note that the nodule is well vascularized (arrow), and there is no evidence of lymphoid cellular infiltration. H & E, × 250. F, low-power magnification of a 9-day nodule. The cells resemble a normal tissue graft and are viable but nonproliferating. H & E, × 63. These photographs should be compared with Fig. 2, which illustrates the progressive growth of the tumorigenic segregant ESH5T in the same animals.
Fig. 2. Histological appearance of the tumorigenic segregant ESH5T injected s.c. into nude mice. A, 24-hr nodule. The nodule has the same appearance as the nontumorigenic ESH5, with central necrosis and a periphery composed of healthy cells. H & E, × 100. B, higher magnification of A. Arrows, dividing cells. H & E, × 250. C, 3-day nodule. The histological appearance of the tumor is identical to that of the nontumorigenic ESH5 (Fig. 1C) with many dividing cells (arrows) present. H & E, × 250. D and E, 5- and 7-day nodules, respectively. The tumor mass is progressively growing, and there is abundant mitotic activity (arrows in D). There has been no morphological alteration of these cells, and the tumor retains the characteristics of a relatively undifferentiated carcinoma. H & E, × 250.
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