Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 7291–7295, July 1994
Medical Sciences

Progressive growth in immunodeficient mice and host cell recruitment by mouse endothelial cells transformed by polyoma middle-sized T antigen: Implications for the pathogenesis of opportunistic vascular tumors

(hemangioma/secondary lesion/nude mice)

C. GARLANDA*, C. PARRAVICINI†, M. SIRONI*, M. DE ROSSI*, R. WAINSTOK DE CALMANOVICI*, F. CAROZZI†, F. BUSSOLINO§, F. COLOTTA*, A. MANTOVANI*¶, AND A. VECCHI*

*Istituto Mario Negri, Via Eritrea, 62, 20157, Milan, Italy; †Ospedale L. Sacco, Milan, Italy; †Centro Immunogenetica Oncologia Sperimentale, Consiglio Nazionale della Ricerche, Turin, Italy; and §Dipartimento di Genetica, Biologia, Chimica Medica, Università di Torino, Turin, Italy

Communicated by Judah Folkman, February 14, 1994 (received for review December 15, 1992)

ABSTRACT **A retroviral construct encoding polyoma middle-sized T antigen was used to generate transformed endothelial cell lines from heart (H5V), brain (B9V), and whole-embryo (E10V) of C57BL/6 mice. When injected into syngeneic recipients, H5V and the less studied B9V and E10V cells caused vascular tumors which, depending on the number of cells inoculated, regressed or progressed, leading to death of the host. When H5V cells were injected into immunodeficient mice, tumors were observed with inocula which did not form lesions in immunocompetent recipients and regression did not occur. Treatment with anti-LFA-1, anti-Thy-1.2, and anti-CD8 antibodies abolished rejection; anti-CD4 was a somewhat less effective inhibitor of resistance. Animals with progressive tumors exhibited secondary lesions in various organs with prominent skin involvement in nude mice. Histologically, the tumors had the appearance of a hemangioma, with areas resembling Kaposi sarcoma. Cells lining vascular lacunae had the morphological features of injected H5V cells. The lesions were characterized by prominent neovascularization and mononuclear cell infiltration. Southern blot hybridization analysis revealed that ≈5% of the cells in the tumor mass were transplanted H5V cells. Thus, the H5V transformed endothelial line causes vascular lesions that are sustained to a large extent by recruitment of host cells and manifests full malignant behavior only in immunocompromised hosts. The hypothesis of a tumor sustained by a minute proportion of transformed cells, which recruit host elements and express full malignant behavior only in immunodeficient hosts, would account for several features of some vascular neoplasms in man.**

Neoplastic processes can directly affect the cellular components of the vessel wall, giving rise to hemangiomas, hemangiosarcomas, and Kaposi sarcoma (KS). Hemangiomas are benign vascular tumors of infancy, which usually regress spontaneously. However, in relatively rare cases, progression of lesions requires treatment which now includes α-interferon (1). KS behaves as an opportunistic tumor, its incidence and aggressiveness increasing in immunodeficient individuals, in particular during human immunodeficiency virus (HIV) infection. The histogenetic origin of KS is still unclear, its cellular components including sheets of endothelial-like cells and spindle-shaped cells with elongated nuclei (for review see refs. 2–5). KS spindle cells can be cultured *in vitro*, and various cytokines, including interleukin 6 (IL-6) and oncostatin M as well as the HIV-1 Tat protein, can stimulate their growth (6–11). When injected into nude mice, KS spindle cells cause vascular tumors by recruitment of host elements (12).

The polyoma virus causes various tumor types including hemangiomas in mice (13). Mice transgenic for the polyoma oncogene encoding the middle-sized T antigen (PmT) show vascular tumors (14, 15) and retroviral constructs with PmT transform murine endothelial cells (ECs) *in vivo* and *in vitro* (16). PmT-transformed ECs injected into mice cause hemangiomas by recruiting host ECs via as yet undefined mechanisms (17). An altered balance between urokinase-type plasminogen activator and its inhibitor(s) may underlie the capacity of PmT-transformed ECs to form abnormal blood vessels (lacunae) (18) whose fully neoplastic nature would thus be questionable.

In the context of our interest in the interplay of cytokines with vascular elements (for review see ref. 19), we have generated PmT-transformed EC lines in C57BL/6 mice. We observed that PmT-transformed ECs cause lesions consisting mostly of recruited host cells and behave as opportunistic vascular tumors exhibiting malignant behavior in immunodeficient hosts. As such, they represent a murine model for opportunistic vascular tumors such as KS and suggest a hypothesis for the pathogenesis of these diseases.

MATERIALS AND METHODS

Generation and Characterization of EC Lines. Various organs were taken from C57BL/6 fetuses at day 15 of gestation and trypsinized. About 10^6 cells in 2 ml were cultured in 12-well plates (Falcon, Becton Dickinson) for 24 hr in Dulbecco's modified Eagle's medium with 10% fetal bovine serum (HyClone), and then cell monolayers were exposed for 2 hr to ≈10^5 neomycin-resistant-colony-forming units of the retrovirus vector N-TKmT per well (ref. 16; gift of E. Wagner, Institute for Molecular Pathology, Vienna). Medium was changed and cells were cultured for 72 hr and then selected with the neomycin analog G418 at 800 μg/ml. Growth of G418-resistant cells was observed after 3 weeks. G418 was used for an additional month, and then cells were cultured without it. Six months later, cells were still completely resistant to G418. Stable lines were obtained from whole embryo (line E10V), heart (line H5V), and brain (line B9V). Chromosome analysis of the H5V line revealed that the cells were of female origin. Expression of PmT-encoded antigen was assessed with a specific polyclonal antiserum (courtesy of Brian

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: PmT, polyoma oncogene encoding middle-sized T antigen; EC, endothelial cell; KS, Kaposi sarcoma; mAb, monoclonal antibody; MST, median survival time; IL, interleukin; TNF, tumor necrosis factor.

¶To whom reprint requests should be addressed.

Schauffhausen, Tufts University, Boston) by immunoprecipitation and immunocytochemistry. Cells were routinely screened for mycoplasma contamination. For fluorescent labeling, cell monolayers were incubated with fluorescent acetylated low density lipoprotein (LDL) (Biomedical Technologies, Stoughton, MA) at 10 μg/ml for 4 hr at 37°C in medium. Alkaline phosphatase was evaluated by a cytochemical assay (20). Class II major histocompatibility antigen expression was determined with an anti-I-A^b monoclonal antibody (mAb) [25-9-3S, from the American Type Culture Collection (ATCC)]. Acetone-fixed Cytospin preparations were stained for factor VIII-associated antigen with a rabbit antiserum (Dakopatts, Glostrup, Denmark) directed against human ECs but crossreacting with mouse ECs. Staining of resting or tumor necrosis factor (TNF)-treated (100 units/ml for 4–24 hr) H5V cells with mAb MEC 13.3 (anti-CD31) (21), mAb 21KC10 (anti-E selectin, courtesy of M. Hahne, Max Planck Institute for Immunology, Freiburg, Germany), affinity-purified 903 rabbit serum (anti-P selectin, courtesy of M. Hahne), mAb MK/1.9 [anti-vascular cell adhesion molecule 1 (VCAM-1), from ATCC] was evaluated by ELISA and/or flow cytofluorometric analysis.

Tumor Transplantation. C57BL/6NCrlBR and Crl:nu/nu(CD-1)BR mice (Charles River Italia, Calco, Italy; usually 10 per group) were given s.c. injections of graded numbers of trypsinized cells. In some experiments, C57BL/6 mice were x-irradiated (4 Gy) 24 hr before tumor injection. The following rat mAbs were used: anti-mouse Thy-1.2 (Y19, courtesy of G. Forni, Centro di Immunogenetico ed Istocompatibilitá, Turin, Italy); anti-mouse CD8 (53-6-72 from ATCC); anti-mouse CD4 (GK1.5 from ATCC); anti-mouse Mac-1 α chain (M1/70.15.11.5 from ATCC), and anti-mouse LFA-1 α chain (M17/4.2 from ATCC). Rat immunoglobulin concentration in ascites was measured by a radial immunodiffusion kit (The Binding Site, Birmingham, U.K.). Mice were treated with 1 mg of mAb divided in three injections (0.2 ml) on days 0 (i.v.) and days 1 and 2 (i.p.). Animals of the control group were injected with saline. Conventional histological specimens from tumors grown in normal or immunodeficient mice were read by one of us after coding. Mice were checked three times a week for tumor appearance and were autopsied at death to look for secondary lesions. Survival was checked every day and is reported as median survival time (MST) in days. Results presented are representative of two to four experiments performed.

Southern Blot Analysis. Tumors were minced and washed extensively to remove contaminating blood. DNA extraction and Southern analysis were performed by established procedures. As a reference population we used *in vitro* cultured H5V endothelioma cells mixed with various numbers of freshly isolated thymic cells. The DNA was digested with restriction endonuclease *Bam*HI, which cuts the retroviral construct at a single site (16). Filters were hybridized with an *Eco*RI fragment containing the PmT sequence (2000 bp) of the probe designated pXTKmT, labeled by random priming. This combination of restriction enzyme (*Bam*HI) and anti-PmT probe resulted in two bands (18 and 7.5 kb) of hybridization with *in vitro* cultured H5V cells.

RESULTS

Generation of C57BL/6 Transformed EC Lines. With a retroviral vector carrying PmT, we established three lines from embryonal heart (H5V), embryonal brain (B9V), and whole embryo (E10V) of C57BL/6 mice. The three lines showed a cobblestone morphology at confluency and the cells maintained a monolayer structure without overgrowth. Transmission electron microscopy revealed that H5V cells (but not B9V and E10V cells) contained intracytoplasmic, osmiophilic multilaminated bodies (see Fig. 3). H5V, E10V, and B9V cells all were uniformly negative for the presence of Weibel–Palade bodies and for budding retrovirus particles. H5V, B9V, and E10V cells showed rapid uptake of fluoresceinated acetylated low density lipoprotein and staining for alkaline phosphatase, but not staining for factor VIII-related antigen. Moreover, they expressed CD31 constitutively and E-selectin, P-selectin, and VCAM-1 following exposure to TNF; they responded to inflammatory cytokines (IL-1 and TNF) with increased production of cytokines (IL-6 and chemokines), procoagulant activity, platelet-activating factor, and adhesion molecules (19, 22). The doubling time of H5V cells during the logarithmic phase of growth was 16 hr. The cells did not express class II major histocompatibility antigens. Chromosome analysis revealed that H5V cells were of female origin and aneuploid.

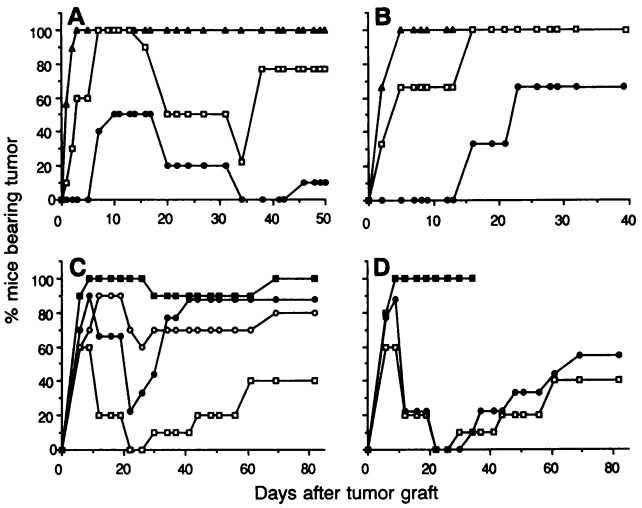

FIG. 1. Tumorigenicity of H5V cells in normal and immunodeficient C57BL/6 mice. (*A*) Tumorigenicity of graded doses of H5V cells in normal mice. The MST of mice (20–40 per group) injected with 2×10^5 (●), 1×10^6 (□), or 5×10^6 (▲) cells was 205, 75, and 55 days, respectively. (*B*) Tumorigenicity of H5V cells in nude mice. The MST of mice (8–10 per group) injected with 2×10^5 (▲), 4×10^4 (□), or 8×10^3 (●) cells was 49, 66, and 68 days, respectively. (*C*) Effect of anti-T cell mAb on the tumorigenicity of H5V cells (2×10^5, given s.c. on day 0). The MST of mice treated on day 0, 1, and 2 with anti-Thy-1.2 (○), anti-CD8 (■), anti-CD4 (●), or saline (□) was 69, 61, 76, and 160 days, respectively. (*D*) Effect of anti-β_2 integrin mAbs on the tumorigenicity of H5V cells (2×10^5, given s.c. on day 0). The MST of mice treated on days 0, 1, and 2 with anti-LFA1 (■), anti-Mac 1 (●), or saline (□) was 22, 122, and 160 days, respectively.

Table 1. Tumorigenicity of H5V cells in normal and immunocompromised hosts

Host	Secondary lesions*	Dead with tumor/total	MST, days (range)
Normal	9/11	6/20†	205 (92–330)
Nude	7/9	9/9‡	49 (40–61)‡
X-irradiated	5/6	8/10‡	160 (80–192)
Anti-Thy-1.2	6/6	8/9‡	69 (22–138)‡
Anti-CD8	19/20	20/20‡	61 (49–126)‡
Anti-CD4	8/9	8/9‡	76 (50–103)‡
Anti-LFA-1	9/10	10/10‡	22 (19–37)‡
Anti-Mac-1	4/4	6/9	122 (69–183)

C57BL/6 or nude mice were given s.c. injection of 2×10^5 H5V cells on day 0.

*No. of mice with secondary lesions over no. of mice that could be autopsied. Secondary lesions, confirmed by histology, were detected in liver, spleen, and gonads. In addition, immunodeficient nude mice showed multiple skin deposits (see also Fig. 1*A*).

†Data of two experiments are pooled.

‡$P < 0.05$ by Fisher or Mann–Whitney test relative to normal C57BL/6 mice.

Tumorigenicity. High doses of H5V cells (5×10^6) caused vascular tumors in female C57BL/6 mice in 2–3 days and killed 100% of the mice with a MST of 55 days (Fig. 1*A*). The tumor induced by 10^6 cells grew for the first 10 days, then regressed and by day 20 disappeared in 80% of the mice. After 35–40 days, regrowth of the tumor was observed in a substantial proportion (80%) of the mice. This picture is reminiscent of the transient retardation of growth concomitant with transient development of effective immunity observed in many tumors (23). With 2×10^5 cells, tumors appeared in only 50% of the mice and then underwent regression. In 30% of the mice a regrowth phase was observed also with inoculum of the 2×10^5 cells. Attempts to transplant the H5V vascular tumor in syngeneic recipients by using disaggregated lesions during the initial growth and rejection phase failed. In contrast, tumors from the mice that exhibited regrowth were transplantable and one line is now at its 11th passage. At necropsy of mice exhibiting progressive growth, secondary lesions were found in several organs (Table 1) including spleen, liver and, most prominently, uterus and ovaries. In ≈40% of the animals, evidence of internal hemorrhage was observed. The H5V tumor showed the same pattern of growth and rejection in male and female mice.

Role of Immunity. Immunocompetent mice that had completely rejected 10^6 H5V cells resisted a second high dose of H5V cells (5×10^6), whereas 6/6 control animals died within 60 days. Immune mice showed no resistance toward the unrelated B16F1 melanoma (data not shown).

To further investigate the role of the immune system, we compared the tumorigenicity of 2×10^5 H5V cells in immunocompetent syngeneic mice and in immunodepressed (irradiated or nude) mice. As shown in Fig. 1*B* and Table 1, 2×10^5 H5V cells caused tumors in all nude mice, whereas they did so in only 30–40% of control, immunocompetent mice. Moreover, while in most immunocompetent mice H5V tumors underwent regression, progressive lesions were observed in all nude mice, which died with a MST of 49 days (Fig. 1*B*). Similarly, an inoculum of 4×10^4 cells, nontumorigenic in immunocompetent mice, caused tumors in nude recipients (Fig. 1*B*). Increased aggressiveness of H5V cells was also observed in x-irradiated hosts (Table 1). Secondary lesions involving the skin were also apparent in nude recipients (Table 1 and Fig. 2*A*). Subcutaneous inoculation of malignant, invasive, and disseminating tumors (B16 and 3LL) did not result in secondary involvement of the skin, thus excluding leakage of cells from the primary injection site.

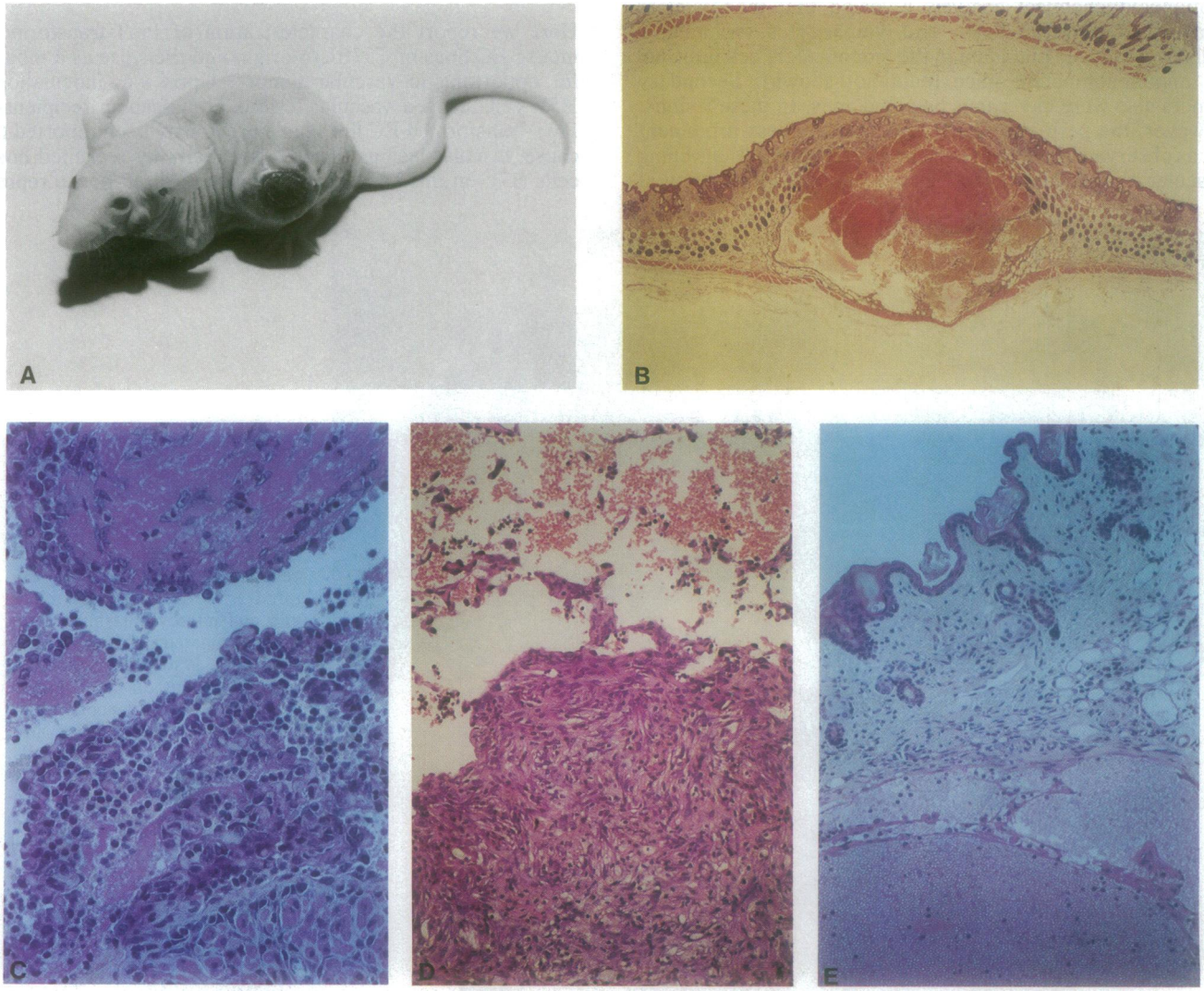

FIG. 2. Morphology of tumors caused by H5V cells. (*A*) Primary and secondary H5V lesions in a nude mouse (4×10^4 H5V cells given s.c., day 30). Secondary skin lesions (confirmed by histology) are visible on the cheek and back. (*B*) Histology of an H5V tumor in a nude mouse (day 14). (Hematoxylin and eosin staining, ×2.3.) (*C*) Prominent mononuclear cell infiltration in a primary H5V tumor in an immunocompetent C57BL/6 host. (Hematoxylin and eosin, ×9.) (*D*) KS-like reaction in the wall of primary angiomatous lesions in a nude mouse. (Hematoxylin and eosin, ×23.) (*E*) Lack of prominent inflammatory reaction in an H5V tumor grown in a nude mouse. (Hematoxylin and eosin, ×9.)

In an effort to initially define leukocyte populations involved in resistance, the effect of specific mAbs was studied. Anti-Thy-1.2 and anti-CD8 mAbs completely abrogated rejection of H5V cells (Fig. 1*C* and Table 1). An anti-CD4 mAb caused a reduction of rejection and an increased regrowth of vascular lesions, though it was a somewhat less effective inhibitor than anti-CD8. Treatment with anti-Mac-1 mAb did not abrogate resistance against H5V cells, whereas treatment with anti-LFA-1 mAb dramatically increased the mortality and abolished resistance to H5V cells (Fig. 1*D* and Table 1).

Histopathology. Primary s.c. tumors had histopathological features consistent with cavernous hemangioma, showing large, blood-filled sacs lined by mono- or plurinucleated cells, arranged in papillary projections or trabeculae (Fig. 2*B*). The tumor appeared to be irregularly encapsulated by a highly vascularized inflammatory reaction involving lymphocytes and monocytes and resembling granulation tissue (Fig. 2*C*). As observed occasionally in human hemangiomas (1), there were spindle-cell areas with KS-like histological appearance (Fig. 2*D*). The inflammatory reaction, assessed blind after coding of samples, was more prominent in immunocompetent than in immunodeficient mice (Fig. 2 *C* and *E*). Rejection of the tumor eventually resulted in the formation of a typical granulation tissue. Electron microscopy and preliminary immunocytochemical analysis revealed that mononuclear phagocytes, myofibroblasts, and lymphocytes, including T cells, were indeed abundant in the mononuclear cell infiltrate of immunocompetent mice (data not shown). Secondary tumors also were cavernous hemangiomas. In these lesions, however, the peritumoral inflammation was less prominent or, as observed in spleen, testis, and liver, absent. Cells lining vascular spaces of primary lesions showed ultrastructural features similar to those found in the cultured cell lines expressing the PmT antigen (Fig. 3*A*). In particular, most cells lining vascular lacunae (Fig. 3*B*) had the same prominent multilaminated cytoplasmic bodies as found in cultured cells. This ultrastructural hallmark, as well as expression of PmT antigen, was absent from the endothelium of recruited blood vessels present in the inflammatory component of the tumor. On the contrary, factor VIII-associated antigen was detectable on endothelial cells of vessels in normal tissues and tumor capsule, but not on cells lining angiomatous blood-filled spaces. In both primary and secondary lesions, type C retrovirus budding particles were observed in cells lining vascular spaces of the tumor as well as in endothelial and fibroblastic cells of tumor capsule (Fig. 3*C*), a phenomenon more prominent in nude mice.

Cell Composition. To estimate the proportion of tumor cells that were derived from transplanted H5V cells, Southern blot experiments were performed using H5V cells mixed with various numbers of normal thymocytes. Fig. 4, representative of four experiments performed, shows a faint but unequivocal hybridization band, corresponding to a frequency of ≈5% H5V-derived cells in the tumors.

DISCUSSION

Here we report the characterization of PmT-transformed mouse EC lines of C57BL/6 origin and their use as a model for opportunistic vascular tumors. Upon s.c. inoculation, H5V cells caused vascular lesions in syngeneic recipients. PmT-transformed EC lines have previously been reported to cause vascular lesions formed essentially by recruited host cells (17): on this basis these lesions appeared not to repre-

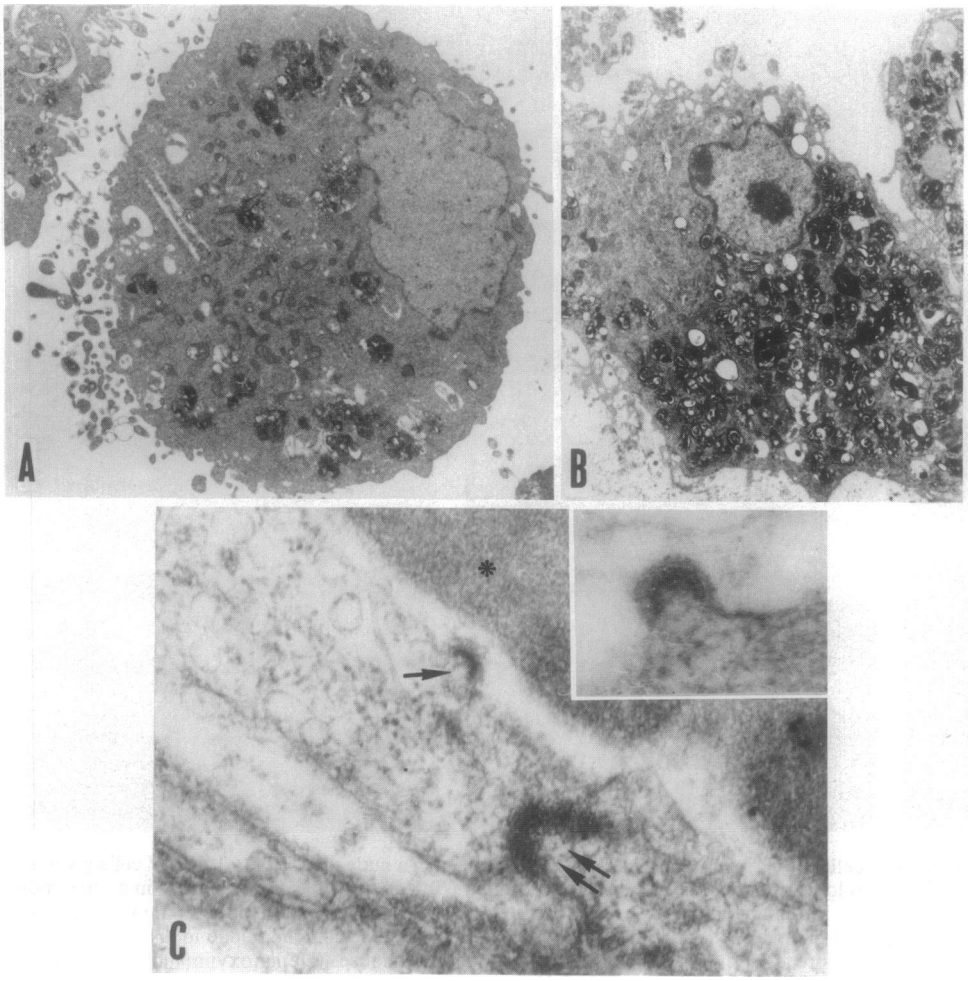

FIG. 3. Morphology of H5V cells *in vitro* and *in vivo*. (*A*) Cultured H5V cells showing microvilli and intracytoplasmic osmiophilic laminated structures consistent with lysosomal myelinoid bodies. (Electron micrograph, ×3200.) (*B*) Subcutaneous tumor in a nude mouse 49 days after injection of 2×10^5 H5V cells. Cells lining blood-filled tumor spaces are characterized by lysosomal myelinoid bodies. (Electron micrograph, ×2200.) (*C*) Virus production in C57BL/6 mice. Virus budding (arrow) in EC of a capillary vessel of the tumor capsule. The double arrow indicates a tight junction between two adjacent EC. The asterisk indicates a circulating erythrocyte. (Electron micrograph, ×9100.) (*Inset*) In the same case virus budding was also present in fibroblastic cells of the interstitium. (Electron micrograph, ×9100.)

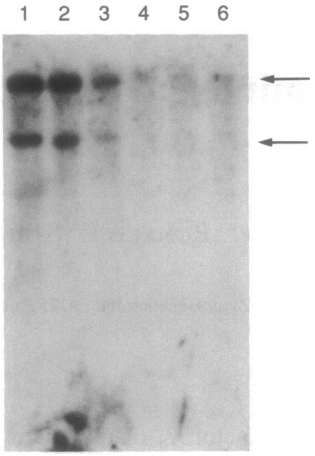

FIG. 4. Southern analysis of PmT in H5V primary tumors. DNA from *in vitro* grown H5V cells was mixed in various proportions (lane 1, 50%; lane 2, 30%; lane 3, 10%; lane 4, 5%) with normal DNA from thymocytes. Lanes 5 and 6 represent two primary tumors. Arrows, 18- and 7.5-kb fragments observed with cultured H5V cells.

sent truly malignant outgrowths. In the present study, the behavior of vascular tumors depended on the inoculum size and, most prominently, on the immunological status of the host. Progressive tumor growth was observed in immunodeficient (nude, irradiated, or anti-T cell antibody-treated) mice, even at low cell doses ($\leq 2 \times 10^5$). Similar results were obtained with B9V and E10V lines. Thus, these transformed ECs cause truly neoplastic lesions which fully manifest their malignant potential only in immunocompromised hosts.

Previous analysis of PmT endotheliomas using nuclear labeling and antimitotic treatment led to the conclusion that PmT-transformed cells cause tumors by recruiting host cells (17). These results were confirmed and extended in the present study. By Southern analysis it was found that H5V cells represent ≤5% of the cellular components of the tumor mass. Yet, this minor proportion of transformed cells was able, particularly in immunodeficient hosts, to sustain "tumor" growth and led to death of the hosts.

The mechanism of *in vivo* recruitment of host vascular cells and leukocytes in H5V tumors remains unclear. In previous analysis a role of virus production was excluded (17). H5V cells cultured alone or with inflammatory peritoneal exudate cells showed no evidence for virus production as shown by electron microscopy and focus formation; the supernatant of cultured cells or the serum of H5V tumor-bearing mice did not cause tumors (data not shown). However, production of particles resembling C-type retroviruses was observed *in vivo*. Thus, we cannot completely exclude that virus production, somehow activated in the *in vivo* microenvironment, contributes to recruitment. PmT-transformed ECs, including those characterized here, produce various cytokines such as IL-6, chemokines, and an ≈40-kDa factor that is chemotactic for vascular cells (19, 22, 24). It is therefore likely that cytokine production plays a pivotal role in leukocyte and EC recruitment.

KS is a skin tumor with prominent vascular involvement whose incidence and aggressiveness are dramatically increased in immunodeficient individuals. The nature of KS (true neoplasia versus hyperplasia) remains uncertain (2, 5). Cell recruitment, including recruitment of vascular cells and leukocytes, is probably important in the pathogenesis of KS lesions, as illustrated by transplantation of spindle-cell cultures in nude mice (12). Although H5V lesions show a prominent and unique tropism for the skin in nude mice (see Fig. 2*A*), the histology of H5V tumors is clearly distinct from that of KS, resembling more that of hemangiomas. Yet the pathogenesis of H5V lesions, which are caused by a ≤5% of transformed cells that recruit normal vascular and leukocytic cells and express full malignant potential only in immunocompromised mice, provides an alternative hypothesis for the pathogenesis of KS. The concept of a tumor sustained by a minute proportion of transformed cells, which recruit host elements and express malignant behavior only in immunodeficient hosts, would explain the difficulty in defining unequivocally, histologically or *in vitro*, the malignant nature of KS, as well as its behavior as an opportunistic aggressive disease associated with immunodeficiency.

This work was supported by the National AIDS Project, the European Community (BIOT-CT90-0195), and Associazione Italiana della Ricerca sul Cancro. C.G., M.D.R., and R.W.C. are recipients of fellowships from Associazione Italiana della Ricerca sul Cancro, Dompé, and the European Community, respectively.

1. Ezekowitz, R. A. B., Mulliken, J. B. & Folkman, J. (1992) *N. Engl. J. Med.* **326**, 1456–1463.
2. Weiss, R. A. (1990) *Eur. J. Cancer* **26**, 657–659.
3. Gottlieb, G. J. & Ackerman, A. B. (1988) in *A Text and Atlas*, eds. Gottlieb, G. & Ackerman, A. (Lea & Febiger, Philadelphia), pp. 73–111.
4. Rosai, J. (1988) in *A Text and Atlas*, eds. Gottlieb, G. & Ackerman, A. B. (Lea & Febiger, Philadelphia), pp. 3–8.
5. Armes, J. (1989) *Adv. Cancer Res.* **53**, 73–87.
6. Ensoli, B., Nakamura, S., Salahuddin, Z. S., Biberfeld, P., Larsson, L., Beaver, B., Wong-Staal, F. & Gallo, R. C. (1989) *Science* **243**, 223–226.
7. Nakamura, S., Salahuddin, S. Z., Biberfeld, P., Ensoli, B., Markham, P. D., Wong-Staal, F. & Gallo, R. C. (1988) *Science* **242**, 426–430.
8. Miles, S. A., Rezai, A. R., Salazar-Gonzàles, J. F., Vander Mayden, M., Stevens, R. H., Logan, D. M., Mitsuyasu, R. T., Taga, T., Hirano, T., Kishimoto, T. & Martìnez-Maza, O. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 4068–4072.
9. Chandran Nair, B., De Vico, A. L., Nakamura, S., Copeland, T. D., Chen, Y., Patel, A., O'Neil, T., Oroszlan, S., Gallo, R. C. & Sarngadharan, M. G. (1992) *Science* **255**, 1430–1432.
10. Miles, S. A., Martìnez-Maza, O., Rezai, A., Magpantay, L., Kishimoto, T., Nakamura, S., Radka, S. F. & Linsley, P. S. (1992) *Science* **255**, 1432–1434.
11. Ensoli, B., Barillari, G., Salahuddin, S. Z., Gallo, R. C. & Wong-Staal, F. (1990) *Nature (London)* **345**, 84–86.
12. Salahuddin, S. Z., Nakamura, S., Biberfeld, P., Kaplan, M. H., Markham, P. D., Larsson, L. & Gallo, R. C. (1988) *Science* **242**, 430–433.
13. Eddy, B. E. (1982) in *The Mouse in Biomedical Research*, eds. Foster, H. L., Small, J. D. & Fox, J. G. (Academic, New York), Vol. 2, pp. 293–311.
14. Bautch, V. L., Toda, S., Hassell, J. A. & Hanahan, D. (1987) *Cell* **51**, 529–538.
15. Dubois, N. A., Kolpack, L. C., Wang, R., Azizkhan, R. G. & Bautch, V. L. (1991) *Exp. Cell Res.* **196**, 302–313.
16. Williams, L. R., Courtneidge, S. A. & Wagner, E. F. (1988) *Cell* **52**, 121–131.
17. Williams, L. R., Risau, W., Zerwes, H.-G., Drexler, H., Aguzzi, A. & Wagner, E. F. (1989) *Cell* **57**, 1053–1063.
18. Montesano, R., Pepper, M. S., Mohle-Steinlein, U., Risau, W., Wagner, E. F. & Orci, L. (1990) *Cell* **62**, 435–445.
19. Mantovani, A., Bussolino, F. & Dejana, E. (1992) *FASEB J.* **6**, 2591–2599.
20. Rambaldi, A., Terao, M., Bettoni, S., Bassan, R., Battista, R., Barbui, T. & Garattini, E. (1989) *Blood* **73**, 1113–1115.
21. Vecchi, A., Garlanda, C., Lampugnani, M. G., Resnati, M., Matteucci, C., Stoppacciaro, A., Schnurch, H., Risau, W., Ruco, L., Mantovani, A. & Dejana, E. (1994) *Eur. J. Cell Biol.* **63**, 247–254.
22. Bussolino, F., De Rossi, M., Sica, A., Colotta, F., Wang, J. M., Bocchietto, E., Martin-Padura, I., Bosia, A., Dejana, E. & Mantovani, A. (1991) *J. Immunol.* **147**, 2122–2129.
23. Di Giacomo, A. & North, R. J. (1986) *J. Exp. Med.* **164**, 1179–1192.
24. Taraboletti, G., Belotti, D., Dejana, E., Mantovani, A. & Giavazzi, R. (1993) *Cancer Res.* **53**, 3812–3816.