opening the M13 construct at the 5′ end of the fragment with an Eco RI digest, filling in recessed termini with the Klenow fragment of *Escherichia coli* DNA polymerase, then releasing the fragment with a Bam HI digest. These fragments were then subcloned directionally into vectors just 5′ of a previously inserted indicator gene, either into the Sma I–Bam HI polylinker sites of a pUC18 vector containing the CAT GenBlock (Pharmacia) in the Hinc II site of the polylinker, or into the Hinc II–Bam HI polylinker sites of a pUC12 vector containing a promoterless hGH gene at the Bam HI–Eco RI sites of the polylinker (*11*). An internal deletion leader fragment which has bases −186 to −151 removed from fragment L1 was generated by performing Bal 31 digestion of L1-CAT from the 5′-most end of the ferritin promoter and extending the digestion into the region corresponding to the 5′ noncoding region of the ferritin mRNA. Release, M13 cloning and sequencing, and subsequent fragment isolation were performed as above. A fragment corresponding to base pairs −150 to −6 was identified, isolated, and subcloned in the proper orientation into L4-CAT at polylinker sites between the L4 fragment and the CAT GenBlock. The net effect was the construction of L1-CAT with an internal deletion of base pairs −186 to −151.

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20. CAT assays on transient transfectants was performed as described (*8*). Assays for hGH production were performed as follows. Murine B6 fibroblasts were transfected in triplicate with 30 μg of plasmid DNA by calcium phosphate precipitation (*8*) and washed twice with media after 16 hours. Transfectants were then cultured at 37°C for 24 hours in Dulbecco's modified essential media before changing to fresh media and adding hemin (100 μ*M*) or desferrioxamine (100 μ*M*) or leaving as untreated control. The time of addition of agents was designated time 0, and aliquots of media were taken and stored at −20°C. Additional aliquots were taken at time points 4, 10, and 24 hours. Levels of hGH as a function of time and treatment were then determined by radioimmunoassay (Nichols Institute, San Juan Capistrano, CA), and the absolute ^{125}I counts as determined by radioimmunoassay plotted as a function of time.
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Phagocytosis of *Candida albicans* Enhances Malignant Behavior of Murine Tumor Cells

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Murine tumor cells were induced to phagocytize either *Candida albicans* or group A streptococcal cells. The presence of microbial particles within the tumor cell cytoplasm had no effect on in vitro tumor cell growth. However, when *Candida albicans*–infected tumor cells were injected into syngeneic mice, they formed tumors that grew faster, invaded the surrounding normal tissue more rapidly and metastasized more rapidly than control tumor cells. Tumor cells infected with group A streptococcal particles did not grow faster or show increased malignant behavior. These data indicate that the in vivo behavior of malignant tumor cells can be modulated by microbial particles, which are often present in the microenvironment of the growing tumor.

PHAGOCYTOSIS OF PARTICLES THE size of red blood cells, mammalian cell nuclei, and microorganisms can be experimentally induced in nonleukocytic cells by a variety of manipulations (*1–4*). Routine histological and cytological examination of tissues and cells taken from human tumors also frequently shows the presence of intracytoplasmic particles (*5–11*), suggesting that this phenomenon is not just an in vitro artifact. The presence of intracytoplasmic bacteria in hyperplastic and dysplastic gut epithelium of several species has been observed and viable bacteria have been isolated from the epithelial cells (*12–20*). These findings suggest that some forms of adenocarcinoma of the ascending colon may have an infectious component to their etiologies (*12, 13*). How the presence of intracytoplasmic infectious agents might influence the behavior of the host cell is difficult to assess because there have been no experimental models with which to study this phenomenon. In the present study, we have induced the intracytoplasmic accumulation of microorganisms by murine tumor cells and have examined the effect on the in vivo biological properties of the cells.

A line of murine fibrosarcoma cells designated as 1.0/L1 was used in our studies. These cells were isolated in our laboratory from a 3-methylcholanthrene–induced tumor in a C57B1/6 mouse (*21*). They grow in monolayer culture on RPMI 1640 culture medium supplemented with 10% fetal bovine serum. When injected subcutaneously into syngeneic mice, they form progressively growing tumors that invade locally and metastasize to the lungs. Micrometastases first become visible at approximately 30 to 40 days after subcutaneous tumor injection, at which time the primary tumors have reached a mean diameter of 10 mm or more.

Phagocytosis of microorganisms [heat-killed group A streptococci (strain C203S, M protein–negative) or *Candida albicans*] by the murine tumor cells was induced as described (*2*). This procedure involved preparing suspensions of heat-killed cells at 1×10^7 cells per milliliter of phosphate-buffered saline (PBS) and opsonizing the microbial cells by the addition of 1 mg of histone per milliliter (type II-A, Sigma Chemical Co., St. Louis, Missouri). After 30 minutes of incubation, the microbial cells were washed repeatedly to separate them from the unbound histone. The opsonized microorganisms were then added to monolayers of tumor cells in serum-free RPMI 1640 medium at a ratio of 10:1 for the *C. albicans* and 50:1 for the streptococci. Although unopsonized microorganisms did not attach to the tumor cells, the histone-treated cells rapidly attached. More than 95% of the added cells were firmly attached to the tumor cell monolayer within 15 minutes. It could be seen microscopically that the opsonized cells did not attach to the plastic dish itself. Morphometric techniques were used to determine the kinetics of microbial cell uptake by the tumor cells and to quantitate the distribution of microbial cells within individual tumor cells. Uptake of the opsonized microbial particles began within 6 hours after being added to the tumor cell monolayer and was essentially complete by 36 hours. Approximately 70% of the tumor cells contained intracellular particles ranging in number from 1 to 10 yeast particles per cell and 1 to 25 bacterial particles per cell.

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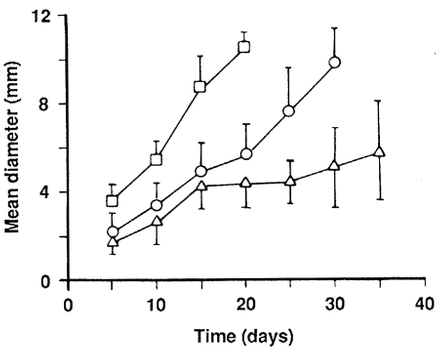

Fig. 1. In vivo growth of control 1.0/L1 murine cells (circles), *C. albicans*–infected tumor cells (squares), and group A streptococci–infected tumor cells (triangles). Six animals in each group were injected in the flank with 1×10^5 tumor cells on day zero. At various times later, the animals were examined for the presence of visible tumor. Tumor diameters were measured in three dimensions with a caliper, and the mean diameter was determined. The values shown are averages plus or minus standard deviations. The experiment was repeated four times with similar results.

The uptake of opsonized microbial cells did not alter the in vitro growth of the tumor cells. This was determined by harvesting the tumor cells 1 day after addition of the microorganisms and replating them in culture medium (RPMI 1640 medium supplemented with 10% fetal bovine serum). The cells containing intracytoplasmic yeast or bacterial particles attached and spread at identical rates to control cells (more than 95% of the cells from all three groups attached and spread within 4 hours). When the replated cells were allowed to grow for 1 or 2 days and then counted, the number of cells recovered from all three groups was similar. The doubling time of the cells from all three groups was 16 to 20 hours, consistent with earlier work (*21*). After each division, there were fewer microbial particles per cell, indicating that the internalized particles were randomly segregating at each cell division.

Although the presence of intracytoplasmic microbial particles did not alter in vitro growth of the tumor cells, the in vivo behavior of these tumor cells was affected. Specifically, *C. albicans*–infected tumor cells produced tumors in the flanks of syngeneic mice that appeared sooner and grew more rapidly than tumors produced by uninfected control cells (Fig. 1). Increased tumor growth depended on internalization of the yeast cells. Injection of unopsonized or opsonized particles along with the tumor cells produced no alteration in tumor growth. The simultaneous injection of histone along with the yeast particles and tumor cells also did not affect tumor growth. Furthermore, when mice were injected with uninfected tumor cells on one side and *C. albicans*–infected tumor cells on the other, the infected tumor cells produced tumors that grew at the rapid rate while the uninfected cells produced tumors that grew at the same rate as tumors induced by uninfected tumor cells in control mice. In contrast to what was observed with *C. albicans*–infected tumor cells, enhanced tumor growth did not occur when group A streptococci–infected tumor cells were used as inoculum (Fig. 1).

Animals were killed for histological examination of the primary tumors at various times after injection. Examination of the primary tumors induced by untreated 1.0/L1 cells and by cells with intracytoplasmic *C. albicans* particles 5 days after injection revealed significant differences (Fig. 2). The tumors induced by the *C. albicans*–infected cells showed a marked polymorphonuclear leukocytic response that was associated with a widespread invasion of the normal tissue surrounding the tumor. In contrast, there was much less inflammation in lesions induced by control tumor cells, and these tumors did not show evidence of invasion at this stage. Differences in tumor size at very early time points may have been due, in part, to differences in the amount of inflammation present. Inflammation is unlikely to account for the entire difference, however, since many more tumor cells were seen in lesions induced by the *C. albicans*–infected tumor cells than in lesions induced by untreated tumor cells.

Except for the tumor size, the intense inflammatory response, and widespread invasion, there was nothing to distinguish the lesions histologically. We saw no evidence, for example, that the vasculature of the two types of lesions was different. Like the control tumors, there was no leukocytic infiltration in tumors induced by group A streptococci–infected tumor cells or in tumors induced by control tumor cells in the presence of opsonized or unopsonized *C. albicans* particles. The presence of inflammatory cells was transient in the lesions induced by the *C. albicans*–infected tumor cells. By day 10, at which time the tumors induced by the *C. albicans*–infected cells had reached 5 to 6 mm in diameter, there was no longer any evidence of significant inflammatory response. The histological picture of the tumors induced by *C. albicans*–infected cells at day 10 was the same as the histological picture of the control tumors at the same stage of development (approximately day 20).

Complete autopsies were done on tumor-bearing animals when the primary tumors reached 10 mm in diameter. For tumors induced by the *C. albicans*–infected cells, this was done at day 20; for control tumors, this occurred on day 30. Histological examination of lung tissue revealed the presence of micrometastases throughout the lungs. Small clusters of tumor cells were visible in both intravascular and extravascular locations. There were no apparent differences in histology or extent of metastatic spread between the animals with control tumors and animals with *C. albicans*–infected tumors. In contrast, when animals with control tumors were examined on day 20, metastatic tumors were not observed in their lungs. Thus, the major difference in metastatic behavior between control tumors and those induced by cells with intracytoplasmic *C. albicans* particles was in the amount of time needed for the development of metastasis.

The findings presented here show that the intracytoplasmic accumulation of microbial particles in tumor cells can have a dramatic effect on the in vivo behavior of these cells. Although the evidence indicates that ingestion of the microbial cells is required for the effects described here (rather than their mere presence at the site of the primary tumor), neither the process of phagocytosis itself nor merely the introduction of histone are sufficient to induce altered behavior. We do not know how the presence of histone-opsonized *C. albicans* particles within the tumor cells facilitates tumor growth. However, this

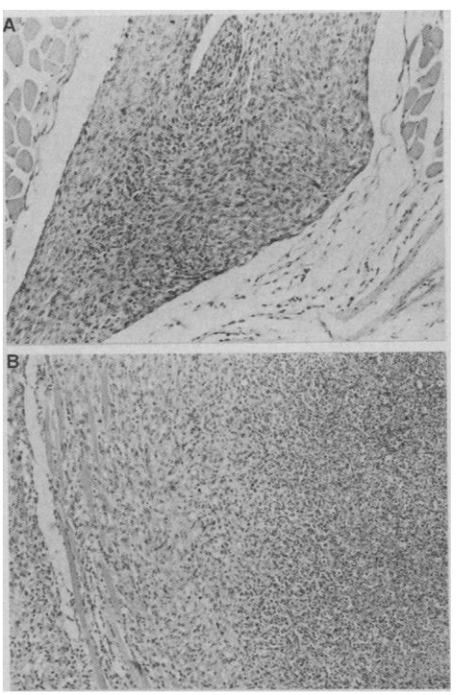

Fig. 2. Histological appearance of tumors induced by uninfected cells and *C. albicans*–infected cells. (**A**) The histological appearance of a tumor 5 days after the animal was injected with uninfected tumor cells. (**B**) Histological appearance of a tumor 5 days after the animal was injected with *C. albicans*–infected tumor cells. (A and B) Stained with hematoxylin and eosin (×50).

does not appear to be a property unique to *C. albicans* since identical results were obtained with another species of candida (*C. glabrata*). We suspect that the initial polymorphonuclear response is in some way involved, but this opinion is based primarily on the dramatic histological differences between the control tumors and those induced by *C. albicans*–infected cells, rather than on an understanding of how the leukocytic infiltrate potentiates tumor growth. The presence of large numbers of leukocytes could facilitate tumor spread by degrading the normal tissue surrounding the tumor. Alternatively, leukocyte products or tumor cell products, or both, could facilitate tumor cell proliferation directly or could enhance formation of the tumor vascular bed. The mechanism underlying the altered in vivo behavior of the *C. albicans*–infected tumor cells is likely to be a local rather than systemic effect since the presence of *C. albicans*–infected tumor cells had no effect on the growth of tumors produced by uninfected tumor cells at distant sites in the same mice. Our work supports the concept that microbial cell infection of tumor cells can alter their behavior, and it provides an experimental model in which to probe the mechanism by which the presence of such particles affects tumor cell behavior as well as the consequences of infection.

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Activation of the HIV-1 LTR by T Cell Mitogens and the Trans-Activator Protein of HTLV-I

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To investigate the mechanism by which immune activation augments replication of the human immunodeficiency virus type 1 (HIV-1) in infected T cells, four different classes of T cell mitogens were evaluated for their effects on the HIV-1 long terminal repeat (LTR). Phytohemagglutinin (PHA), a mitogenic lectin; phorbol 12-myristic 13-acetate, a tumor promoter; ionomycin, a calcium ionophore; and *tat*-1, the trans-activator protein from the human T cell leukemia/lymphoma virus type I (HTLV-I) each stimulated the HIV-1 LTR. Studies of deleted forms of the LTR supported a central role in these responses for the HIV-1 enhancer, which alone was sufficient for mitogen inducibility, but also suggested that other 5′ positive and negative regulatory elements contribute to the overall magnitude of the response. Synergistic activation of the HIV-1 LTR (up to several thousandfold) was observed with combinations of these mitogens and the HIV-1–derived *tat*-III protein. Cyclosporin A, an immunosuppressive agent, inhibited PHA-mediated activation of the HIV-1 LTR but was without effect in the presence of the other mitogens. Thus, HIV-1 gene expression and replication appear to be regulated, via the HIV-1 LTR, by the same mitogenic signals that induce T cell activation.

INFECTION WITH HUMAN IMMUNODEFICIENCY virus type 1 (HIV-1) in vivo may be associated with an asymptomatic interval lasting from months to years before the development of frank disease (*1*). Under selected conditions of culture in vitro, HIV-1–infected $CD4^+$ T cell lines can be maintained for long periods in the absence of detectable virus production (*2*). The events that trigger the transition from low-level or latent infection to productive viral replication remain poorly defined. Immune activation of infected T cells can augment HIV-1 replication (*2, 3*) perhaps through effects on regulatory elements located within the long terminal repeat (LTR) of HIV-1 (*4*). Here we show that four different classes of T cell mitogens [phytohemagglutinin (PHA), phorbol 12-myristic 13-acetate (PMA), ionomycin, and *tat*-1 (*5*)] can activate the HIV-1 LTR and define the cis-acting LTR sequences that mediate these responses. We also examine the interactions of these mitogenic stimuli with each other, with the HIV-1–encoded trans-activator protein (*tat*-III), and with the immunosuppressive agent cyclosporin A (CyA).

To confirm that mitogenic stimulation enhanced HIV-1 gene expression, we performed in vitro RNA transcription assays with HIV-1–infected Jurkat T cells before and after combined stimulation with PHA plus PMA (Fig. 1A). As monitored with *tat*-III complementary DNA (cDNA) (which contains 5′ sequences common to all HIV-1 transcripts), these mitogens induced HIV-1 gene transcription within 4 hours. As expected in activated T cells, these mitogens

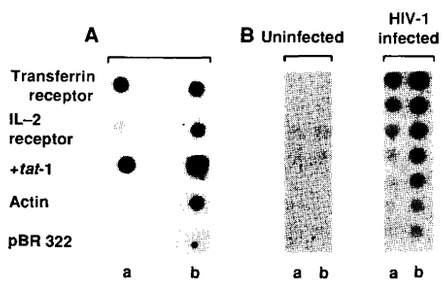

Fig. 1. (**A**) Nuclear transcription analysis of mitogen-induced gene expression in HIV-1–infected Jurkat T cells. Jurkat T cells were infected as described (*2*) and cloned 7 days later. At the time of analysis (after 50 days in culture), no RT activity was detectable. Nuclei were isolated after 4 hours of culture in the (a) absence or (b) presence of PHA (1 μg/ml) plus PMA (25 ng/ml) (*24*). In vitro RNA transcription was performed as described (*25*), using a time point previously shown to be within the linear range for Jurkat cells (*26*). The ^{32}P-radiolabeled nuclear RNA was hybridized to cDNAs immobilized on nitrocellulose filters as described (*26*). (**B**) Dot-blot analysis of HIV-1 RNA levels in infected $CD4^+$ peripheral blood lymphocytes after mitogen activation. $CD4^+$ lymphocytes were purified from normal donors by affinity rosetting, activated with PHA, and infected with HIV-1 (*2*). Cultures were maintained in RPMI 1640 with 10% fetal calf serum and supplemented with 10% partially purified human IL-2 (*24*). Six days after infection, uninfected and HIV-1–infected cultures were washed and incubated with (a) medium or (b) medium supplemented with PHA (1 μg/ml) plus PMA (25 ng/ml) for 18 hours. Total cytoplasmic RNA was isolated, serially diluted twofold, immobilized on nitrocellulose filters (*27*), and hybridized to ^{32}P-radiolabeled *tat*-III cDNA.

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