inefficient selection when transformed cells were near. Examination of four such plants revealed that three contained the expected DNA sequences homologous with pMON200, indicating that loss of foreign gene expression may account for many of the apparent escapes. The problem could be managed easily by first screening for plantlet rooting in the presence of 100 μg of kanamycin per milliliter and then applying the nopaline and leaf callus assays to assure stable expression in plants for further analysis and testing of progeny.

Unexpected changes in expression of T-DNA genes in plant cells and frequent loss of expression of opine synthase genes have been reported (*14*). Induction of genes for opine synthase upon transfer of explants to culture medium has also been observed (*15*). The predictable induction of these genes in certain of our transformants and the transmission of this property to progeny may be due to influences from surrounding DNA or chromatin structure at the site of insertion of the foreign genes into a plant chromosome. Such position effects have been observed frequently in other eukaryotic systems, such as transgenic mice (*16*) and *Drosophila* (*17*).

The leaf disk transformation system should be applicable to many of the plant species that are susceptible to *A. tumefaciens* and can be regenerated from leaf explants. By integrating the transformation, selection, and regeneration processes into a simple and efficient procedure, the production of transformed plants could become routine for studies of gene expression and of the physiology or biochemistry of plants, even in laboratories with little expertise in tissue culture methods.

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9. Leaf disks were incubated on nurse culture plates with medium (*p*H adjusted to 5.7) containing MS salts (Gibco), B5 vitamins, sucrose (30 g/liter), benzyladenine (1.0 μg/ml), naphthalene acetic acid (0.1 μg/ml), and 0.8 percent agar. Shoots were rooted on similar medium lacking phytohormones.
10. The F_1 hybrid petunia was produced by crossing lines V23 and R51. The same media were used for petunia as for tobacco (*9*).
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Phagocytes as Carcinogens: Malignant Transformation Produced by Human Neutrophils

Abstract. *In a study of the relation between chronic inflammation and carcinogenesis, C3H mouse fibroblasts of the 10T 1/2 clone 8 line (10T 1/2 cells) were exposed to human neutrophils stimulated to synthesize reactive oxygen intermediates or to a cell-free enzymatic system generating superoxide (xanthine oxidase plus hypoxanthine). After exposure, the 10T 1/2 cells were either placed in tissue culture or immediately injected into athymic nude mice. Both malignant and benign tumors developed in the mice injected with treated cells, but not in those injected with control cells; in one instance cells grown from one of the benign tumors subsequently developed a malignant phenotype. Malignant transformation was also observed in treated cells in the experiments in vitro.*

We showed previously that human phagocytes can produce mutations in bacteria and mutations and sister chromatid exchanges in cultured hamster ovary cells, and that reactive oxygen metabolites were important for the production of these genetic lesions (*1–4*). The results suggested that the toxic intermediates or by-products of oxygen metabolism generated by inflammatory phagocytes may play a role in the carcinogenic process. The findings of other groups concur with these observations (*5–10*). We conducted the present study to address this question more directly.

The cells used were C3H mouse fibroblasts of the 10T 1/2 clone 8 line (10T 1/2 cells). At passages 10 through 12, 10T 1/2 cells were grown in monolayer culture and treated as shown in Table 1. After the treatment the cells were washed, removed from the petri dishes, and plated according to standard methods for transformation assays (*11–13*). After 6 to

Table 1. Transformation of C3H 10T 1/2 cells in vitro. The C3H 10T 1/2 cell line and transformation assay methods were described previously (*11–13*). Briefly, plateau-phase ($\sim 4 \times 10^4$ cells per square centimeter) cells, grown in Eagle's basal medium containing 10 percent fetal calf serum, were treated while they were attached to plastic 100-mm petri dishes. Neutrophils were prepared from healthy human volunteers as described (*20*). In general, target 10T 1/2 cells were incubated for 60 minutes at 37°C with neutrophils and with or without TPA (Consolidated Midland; 1.0 μg/ml). In some experiments, xanthine oxidase (15 μg/ml) and hypoxanthine (7 μg/ml), both from Sigma, were layered onto the 10T 1/2 cells instead of neutrophils. At the end of the incubation period the 10T 1/2 monolayers were washed, removed from the dishes, and plated into 100-mm dishes at densities such that less than 2000 surviving cells per dish were expected (on the basis of plating efficiency determinations). Medium was changed twice weekly until confluence was reached, then weekly thereafter until fixation and staining were performed at 6 to 8 weeks.

Treatment	Number of cells surviving per dish	Total number of dishes	Dishes with type III foci	
			Total	Percentage
None (control)	1265	210	7	3.3
Hypoxanthine plus xanthine oxidase	1335	20	3*	15.0
Neutrophils (10^6)	1380	20	2	10.0
Neutrophils (5×10^6)	1200	20	10*	50.0
Neutrophils plus TPA	1320	20	7*	35.0
Neutrophils (5×10^6) plus TPA	480	20	3*	15.0
TPA (1 μg/ml)	1005	20	1	5.0

*Significantly greater ($P < .05$) transformation frequency than untreated control (*21*).

Table 2. Tumor development in athymic nude mice inoculated with 10T 1/2 cells treated as described in Table 1. The cells were washed, removed from the dishes, aspirated into syringes, and injected subcutaneously (8×10^5 to 10^6 cells per mouse). The animals were then observed regularly for the appearance of tumors at the injection site.

Treatment	Malignant tumors	Benign lesions	Total number of mice	Number of mice with tumor
None	0	0	32	0
TPA	0	0	21	0
Hypoxanthine plus xanthine oxidase	1	2	10	3
Neutrophils (5×10^6) plus TPA	3	2	22	5
Neutrophils (10^7) PMN plus TPA	1	2	11	3

8 weeks in culture, the cultures were stained and examined for the presence of transformed, type III, foci (*11*). When foci with type III morphology are implanted in genetically appropriate mice, malignant tumors develop in 80 to 90 percent of the recipients (*11, 12*). As shown in Table 1, human neutrophils were able to induce significantly increased numbers of transformants. This transforming effect occurred whether or not the neutrophils were exposed to 12-tetradecanoylphorbol-13-acetate (TPA) during the 1-hour incubation period with the 10T 1/2 cells. The TPA had been added to activate the oxidative metabolism of neutrophils (*3, 7, 8*), but we subsequently observed that the 10T 1/2 cells stimulated the release of superoxide by the neutrophils of two of seven healthy volunteers (including those used in these experiments) directly, without the addition of TPA or other activators.

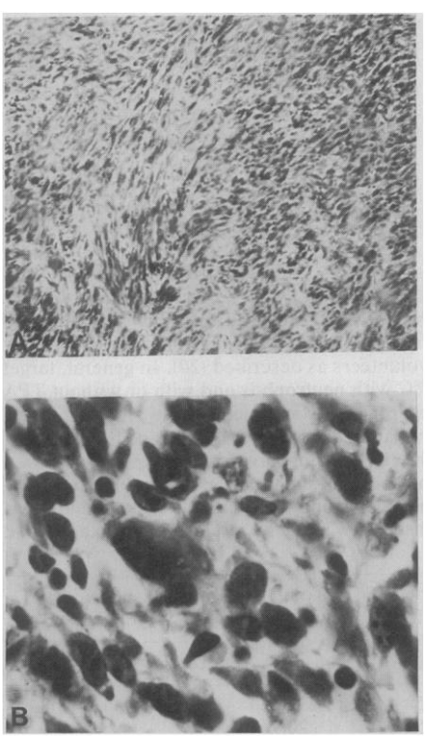

Fig. 1. (A) Low-power (×100) photomicrograph of histologic section of sarcoma from nude mouse, showing dense cellularity. (B) High power (×630) view of same tumor, demonstrating hyperchromatic nuclei and irregular cell size and shape.

Maximum transformation was observed when the 10T 1/2 cells had been incubated with either 10^6 neutrophils plus TPA or with 5×10^6 neutrophils alone. To determine whether reduced oxygen metabolites generated by the phagocytes could be contributing to the transforming activity, we performed experiments in which the enzymatic system generating the superoxide (xanthine oxidase plus hypoxanthine) replaced the neutrophils in incubations with the 10T 1/2 cells. As shown in Table 1, the enzymatic system also induced transformation, confirming a role for reduced oxygen species in the process. Zimmerman and Cerutti recently reported similar findings with xanthine oxidase plus xanthine (*5*).

In a parallel series of experiments we determined whether 10T 1/2 cells implanted into athymic nude mice immediately after treatment in vitro could undergo transformation in vivo and develop into tumors. [Blair *et al.* (*14*) found that freshly transfected NIH 3T3 cells gave rise to tumors in nude mice in 5 to 9 weeks.] As shown in Table 2, tumors developed in nude mice injected with treated cells, but not in 53 control animals (32 injected with untreated 10T 1/2 cells and 21 injected with 10T 1/2 cells treated with TPA alone). The tumors appeared 13 to 22 weeks after injection and were excised and examined histologically; five of the tumors were malignant and six were benign. Four of the malignant lesions were poorly differentiated sarcomas (Fig. 1), and one was not classifiable. The benign lesions were more heterogeneous. One was an angioma with a completely benign histologic appearance (Fig. 2A), one was a fibrous mass that appeared at 18 weeks, grew, and then regressed to about half its maximum size prior to excision, and the remaining four were multiloculated cystic structures of uncertain type (Fig. 2B). Cells from three of the malignant tumors and the benign angioma were successfully placed into tissue culture, where they all grew with typical transformed morphology. At early passages (3 through 5), cells derived from the three fibrosarcomas and from the benign angioma were injected back into nude mice (10^6 cells per mouse). The cell lines derived from the three sarcomas produced identical tumors in 2 to 4 weeks in four out of four, two out of two, and one out of five mice, respectively; the cell line derived from the benign angioma gave rise to malignant sarcomas in 4 to 5 weeks in four out of four mice (Fig. 2C). Cytogenetic stud-

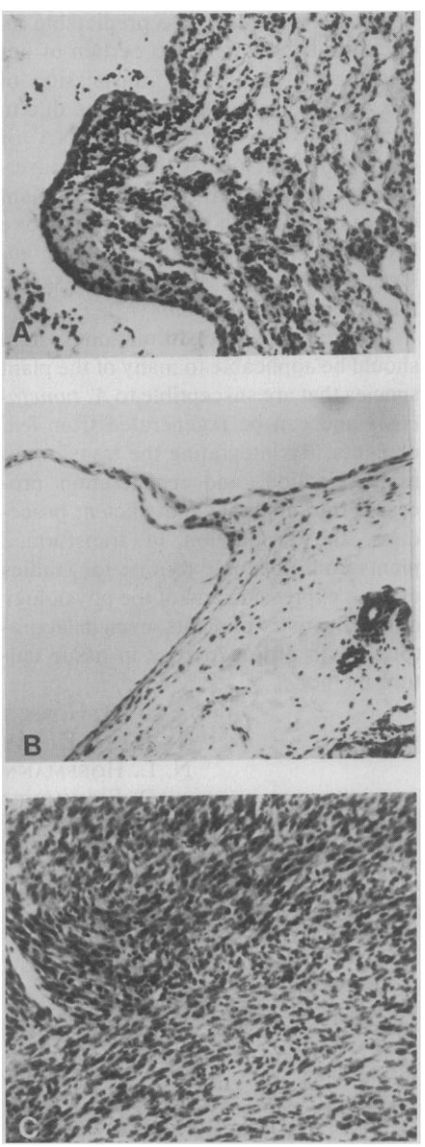

Fig. 2. Low-power (×100) photomicrographs of (A) the benign angioma (see text), (B) one of the multiloculated cystic structures arising at the site of injection of 10T 1/2 cells, and (C) of the sarcoma that arose from cells grown in vitro from the tumor shown in (A) and injected back into a nude mouse.

ies confirmed that the sarcoma-derived cell lines were of murine cell origin.

These experiments demonstrate that inflammatory phagocytic cells can induce malignant transformation. The reasons for the absence of an obvious dose-response effect are not clear, but such an absence has been observed previously (*1, 2, 4*). Some possible explanations are as follows. (i) While at low doses the cells can repair the multiple types of cellular lesions that may be produced by phagocytes, as the dose increases there may be a narrow range within which the cells undergo transformation, with death resulting from any dose above this range. (ii) Some clumping of cells may occur and result in different levels of exposure. (iii) Dead or nonproducing cells may quench or buffer the toxic products. (iv) Cells may vary in their susceptibility to transformation according to their stage in the cell cycle. It is interesting that 5×10^6 neutrophils plus TPA induced fewer transformed foci than 5×10^6 neutrophils alone (Table 1). This suggests that explanations (i) or (iii) are more likely than the others to account for the lack of a clear-cut dose response.

The finding of benign lesions in some of the animals is also of interest. While in some settings transformation in vitro can appear to occur by way of a single step (*15*), in others a multistage model seems more applicable (*5, 16*). The benign angioma observed here, and its subsequent evolution into a malignant cell line, suggests that a process involving more than one step occurred in our experimental system, despite the single brief exposure to the transforming agent.

Although phagocytes stimulated by a variety of agents (bacteria, TPA, opsonized zymosan, exposure to 10T 1/2 cells) to produce oxygen metabolites cause DNA and chromosomal damage and transformation (*1–4, 6–8*), and although a cell-free oxidizing system can have similar effects (*3, 5, 9*), the specific molecular species ultimately responsible for these events are unknown. In addition to producing the superoxide anion, hydrogen peroxide, and the hydroxyl radical, phagocytes produce strongly oxidizing halogenated amines and hypohalous acids (*17–19*). Furthermore, the interaction of these strong oxidants with membrane constituents of both phagocytes and the target cells can generate multiple biologically active or toxic intermediates (for example, peroxides and aldehydes). While earlier work suggests an important role for the hydroxyl radical in the overall process, this compound is so reactive that when it is generated extracellularly it probably cannot reach the target nucleus. However, its reactions might generate other species that can. The ultimate carcinogen remains to be defined.

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Monoclonal Antibodies Against the Aster Yellows Agent

Abstract. *Hybridoma clones secreting specific monoclonal antibodies against the aster yellows agent, a mycoplasma-like organism, were produced by using partially purified salivary gland preparations from infected leafhopper vectors as the immunogen. After 3947 hybridomas from 20 independent fusions were screened for specific antibody against the aster yellows agent, two table clones were obtained. With these monoclonal antibodies the aster yellows agent in diseased lettuce, periwinkles, and inoculative insects was specifically identified by enzyme-linked immunosorbent assay. The aster yellows agent was serologically differentiated from the mycoplasma-like organisms associated with ash yellows, loofah witches'-broom, paulownia witches'-broom, sweet potato witches'-broom, peanut rosette, maize bushy stunt, and elm phloem necrosis.*

Aster yellows (AY), an economically important disease affecting many crops (*1*), is caused by a mycoplasma-like organism (MLO). Although the AY agent was believed for more than 50 years to be a virus (*2*), in 1967 mycoplasmas were implicated as the causal agent of AY and other plant diseases with similar symptoms (*3, 4*). Since then, more than 200 plant diseases have been ascribed to MLO's. The AY agent has been considered to be the most prominent member of the group because it has been implicated in the induction of important diseases of many hosts. During the time in which the agent was thought of as a virus, many unsuccessful attempts were made to purify it (*5*). Even since the discovery that the causative agents were wall-less prokaryotes, attempts to isolate and cultivate these microorganisms have been unsuccessful. To our knowledge, none of the MLO's associated with yellows diseases of plants have been successfully grown in vitro. Thus, in the absence of cultivation of the causative agents, the numerous yellows diseases could be differentiated only by indefinite biological properties such as host range, symptomatology, and insect vector relations.

The AY agent, like other MLO's, inhabits only the sieve tube elements of the phloem tissues of its plant hosts. Since