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.

SIGMUND A. WEITZMAN
ALAN B. WEITBERG
Hematology-Oncology Unit, Massachusetts General Hospital Cancer Center, and *Department of Medicine, Harvard Medical School, Boston, Massachusetts 02114*
EDWARD P. CLARK
Department of Radiation Medicine, Massachusetts General Hospital Cancer Center, and *Harvard Medical School*
THOMAS P. STOSSEL
Hematology-Oncology Unit, Massachusetts General Hospital Cancer Center, and *Department of Medicine, Harvard Medical School*

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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

these sieve elements occupy only a small portion of the plant and are very difficult to separate mechanically from other tissues, there are difficulties in obtaining sufficient quantities of pure AY organisms for use as antigen. Although antigen preparations from plants were used recently to produce polyclonal antisera against the AY and other MLO agents (*6, 7*) that were reactive in the enzyme-linked immunosorbent assay (ELISA), the serological data generated were not always convincing. Such sera have relatively low specific titers and exhibit considerable nonspecific reactions because of the presence of antibody to plant antigens. We now report the successful production of monoclonal antibodies specific to the AY agent, achieved by using insect vector salivary glands as antigen and the sensitive screening method for hybridoma production.

Table 1. ELISA reactions (at 490 nm) of AY monoclonal antibodies from hybridomas 3.2G11 and 3.2F7 with healthy and AY-infected lettuce at different antibody dilutions. The enzyme was avidin-biotinylated peroxidase and the substrate *o*-phenylenediamine. Values greater than 0.1 are considered as being positives.

Reciprocal of culture supernatant dilution	3.2G11		3.2F7	
	Diseased vascular preparation*	Healthy control	Diseased vascular preparation*	Healthy control
1	1.862	0.006	1.979	0.000
100	1.787	0.003	1.802	0.009
200	1.234	0.008	1.432	0.012
400	0.929	0.011	1.069	0.000
800	0.421	0.000	0.403	0.007
1,600	0.129	0.012	0.113	0.000
3,200	0.024	0.015	0.022	0.010
6,400	0.000	0.001	0.006	0.004
12,800	0.012	0.003	0.005	0.013

*Diluted 20 times (weight to volume) in coating buffer.

Table 2. ELISA reactions (at 490 nm) of AY monoclonal antibodies (undiluted culture supernatant) from hybridomas 3.2G11 and 3.2F7 with healthy and AY-infected lettuce at different dilutions of vascular preparation.

Reciprocal of vascular preparation dilution	3.2G11		3.2F7	
	Diseased vascular preparation	Healthy control	Diseased vascular preparation	Healthy control
20	1.851	0.012	2.009	0.016
60	1.777	0.001	1.952	0.003
180	1.495	0.013	1.446	0.005
540	0.494	0.005	0.666	0.007
1,620	0.367	0.002	0.410	0.000
4,860	0.112	0.002	0.170	0.000
14,580	0.070	0.000	0.061	0.006
43,740	0.015	0.009	0.016	0.007
131,220	0.002	0.011	0.013	0.003

Table 3. ELISA reactions (at 490 nm) of AY monoclonal antibodies (undiluted culture supernatant) from hybridomas 3.2G11 and 3.2F7 with healthy and MLO-infected plants.

Vascular preparation of MLO-infected plants	3.2G11		3.2F7	
	Diseased vascular preparation*	Healthy control	Diseased vascular preparation*	Healthy control
Ash yellows	0.017	0.009	0.000	0.004
Loofah witches'-broom	0.005	0.013	0.009	0.008
Paulownia witches'-broom	0.006	0.003	0.012	0.018
Sweet potato witches'-broom	0.006	0.001	0.011	0.013
Peanut rosette	0.007	0.014	0.015	0.010
Maize bushy stunt†	0.013	0.007	0.009	0.003
Elm phloem necrosis	0.004	0.003	0.002	0.006
Aster yellows	1.071	0.005	1.152	0.011

*Diluted 20 times (weight to volume) in coating buffer. †Prepared from corn; all the others are from periwinkle.

The AY agent multiplies and reaches a high titer in the salivary glands of its insect vector (*8*). The antigen used in this study was thus prepared from heads or salivary glands from approximately 2000 inoculative leafhoppers (*Macrosteles fascifrons* Stal) allowed to feed on AY-infected lettuce for 3 days and subsequently maintained on rye plants for 2 weeks to permit completion of the latent period. The dissected salivary glands were homogenized in 0.02*M* phosphate-buffered saline (PBS) and the homogenate was cleared by centrifugation (2000*g*). The partially purified AY agent was then used to immunize 6-week-old female BALB/c mice. Intraperitoneal injections were given on days 1 and 28 and an intravenous injection on day 35, with 200 μg of protein per injection. The mice were killed on day 38. Murine myeloma cells [P3/NS1/1-Ag4-1 (NS1)] were fused with splenic cells from the immunized mice. Hybridomas were selected after incubation in HAT medium (*9, 10*).

A total of 3947 hybridoma clones were produced in 20 independent fusions. Screening of antibody production from hybridomas was performed by indirect ELISA with biotinylated antibody to mouse immunoglobulins G and M (*11*). ELISA plates were coated with insect salivary gland preparations or AY agent concentrated from diseased lettuce plants. The preparation of antigen from salivary glands for ELISA was similar to that used before immunization, except that the homogenate was subjected to differential centrifugation (2,000 and 20,000*g*). The final pellet was diluted to 20 μg of protein per milliliter in 0.05*M* carbonate buffer (*p*H 9.6). To prepare antigen from diseased lettuce, midribs were first dissected from the leaves and cut into 1-cm segments. The tissues were then placed in PBS and subjected to low-speed blending for 2 minutes to loosen and separate the parenchyma cells from the filamentous vascular bundles. Sometimes fine forceps were used to further separate phloem tissues from xylem tissues. These isolated vascular bundles were ground and diluted 20 times (weight to volume) in 0.05*M* carbonate buffer (*p*H 9.6) and then cleared by low-speed centrifugation (2000*g*). Antigen was also prepared from healthy lettuce plants by the same technique.

In the ELISA screening tests, 215 hybridoma clones were positive for both healthy and diseased lettuce, but only five clones produced antibodies specific for AY-diseased lettuce and insects. Two of these five hybridomas (3.2G11 and 3.2F7) survived subculturing and were used for monocloning. Twenty-five

and 30 monoclones were obtained from clones 3.2G11 and 3.2F7, respectively, by the limiting dilution method (*12*). All of these monoclones belonged to the immunoglobulin G3 isotypes, as determined by the Ouchterlony method with antibodies to the mouse isotype.

Monoclonal antibodies were harvested from culture supernatants when cell titers reached 5×10^6 to 7×10^6 cells per milliliter and were used to detect the AY agent in diseased lettuce. As shown in Table 1, the AY agent could be detected when such monoclonal antibodies were diluted up to 1600 times and vascular preparations were diluted 20 times with coating buffer (0.05*M* carbonate buffer, *p*H 9.6). Reciprocally, serial dilutions of lettuce vascular preparation were also used to test the detecting ability of the antibodies. The AY agent could be easily detected when the antigen was diluted 4,860 to 14,580 times (Table 2). Using the same antibodies we found that they also reacted specifically to AY antigen prepared from infected periwinkle plants (*Catharanthus roseus* L.) or inoculative *M. fascifrons*, but not to preparations from healthy periwinkles or noninoculative insects. On the other hand, the AY monoclonal antibodies did not react with vascular preparations of corn infected with maize bushy stunt and from six other MLO-infected periwinkle plants: ash yellows, loofah witches'-broom, paulownia witches'-broom, sweet potato witches'-broom, peanut rosette, or elm yellows (elm phloem necrosis) (Table 3).

Monoclonal antibody–producing hybridoma cells were used to induce ascites by injecting 10^6 cells into pristane-primed BALB/c mice. The ascitic fluids harvested resulted in a 100 to 1000 times higher antibody titer (160,000 to 1,600,000) than those of the culture supernatants (1600).

Until the MLO's that are associated with AY diseases can be cultured, their identification will depend on techniques that are not based on pure antigens. Antibodies developed by our hybridoma technique are reliable for AY diagnosis and for comparison with similar yellows disease agents, providing that antigens can be obtained from them. The technique may be the only means for preparing specific antibodies against these agents because of their apparent noncultivability and their inevitable contamination by plant or insect antigens when they are purified by the techniques of virology. Because of their highly discriminatory capacity, such monoclonal antibodies can now act as a useful tool in yellows disease diagnosis, forecasting, and epidemiology, and they may also be used in taxonomic differentiation among the various MLO's associated with plant yellows diseases.

Note added in proof: Since this report was submitted, five more hybridoma clones have been obtained by using the partially purified MLO preparations from diseased lettuce as immunogen.

CHAN-PIN LIN
TSEH AN CHEN
Department of Plant Pathology, Cook College, New Jersey Agricultural Experiment Station, Rutgers University, New Brunswick 08903

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10. RPMI 1640 complete medium is RPMI 1640 medium supplemented with 15 percent fetal bovine serum. HAT medium is complete medium supplemented with $10^{-4}M$ hypoxanthine, $4 \times 10^{-7}M$ aminopterin, and $1.6 \times 10^{-5}M$ thymidine.
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Coexpression of Translocated and Normal c-*myc* Oncogenes in Hybrids Between Daudi and Lymphoblastoid Cells

Abstract. *Mechanisms that affect the transcription of the* c-myc *oncogene take part in the development of B-cell neoplasias such as Burkitt's lymphoma. Daudi Burkitt lymphoma cells, which express only the translocated* c-myc *oncogene, were hybridized with human lymphoblastoid cells, which express the normal* c-myc *gene; the hybrids were phenotypically lymphoblastoid and expressed both the translocated and the normal* c-myc *gene. This result contrasts with the findings that the decapitated* c-myc *gene, translocated to an immunoglobulin switch* μ *or* α *region, is transcriptionally silent in lymphoblastoid hybrids. Thus, there may be at least two distinct enhancer-like elements capable of deregulating* c-myc *transcription in lymphomas and leukemias with t(8;14) chromosome translocations. In addition, since the Daudi × lymphoblastoid hybrids express both the translocated and the normal* c-myc *gene, the* c-myc *gene product does not autoregulate* c-myc *transcription.*

Approximately 75 percent of Burkitt lymphomas carry t(8;14) chromosome translocations; the remaining 25 percent carry either t(8;22) or t(2;8) chromosome translocations (*1*). In Burkitt lymphomas with a t(8;14) translocation, the c-*myc* gene, which may remain intact or lose a portion of its 5′ end, translocates to various sites within the heavy-chain locus on chromosome 14 (*2*). In the variant t(8;22) and t(2;8) translocations, the c-*myc* oncogene remains on chromosome 8, and either the λ or the κ locus translocates to a region distal (3′) to the c-*myc* gene (*3, 4*). These chromosome translocations result in transcriptional deregulation of the c-*myc* oncogene, causing elevated constitutive expression (*3, 5*). Because the breakpoint near the c-*myc* locus—whether 5′ or 3′ of the gene or close to, within, or distant from it—and the breakpoints within the immunoglobulin loci are heterogeneous, it is difficult to propose a single model for c-*myc* deregulation. An additional complication is that the exact stage of B-cell differentiation represented by Burkitt lymphoma cells is not known and may differ among different tumor lines. In experiments to determine the mechanisms taking part in regulation of c-*myc* expression during B-cell differentiation, we have used somatic cell hybridization methods to construct cells of various phenotypes within the B-cell lineage that carry both translocated and normal c-*myc* genes within the same cell; that is, these hybrids have a Burkitt genotype with a less differentiated lymphoblastoid or more differentiated plasma cell phenotype (*3, 5, 6*).