and fungal-induced wall appositions can be viewed as basically similar plant cell reactions to localized perturbations. See J. R. Aist [*Annu. Rev. Phytopathol.* **14**, 145 (1976)], for a detailed discussion of wall appositions, and P. C. Mercer, R. K. S. Wood, and A. D. Greenwood [*Physiol. Plant Pathol.* **4**, 291 (1974)], for an additional ultrastructural comparison of mechanically and fungal-induced appositions.
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Host Control of Tumor Growth

Abstract. *A humoral factor (molecular weight less than 60,000) that was present in the ascitic fluid of mice bearing intraperitoneal tumors and in pleural effusions from human cancer patients was found to promote the growth of a murine tumor and to suppress cell-mediated tumor immunity. However, the hosts that had recovered from the immunosuppressive state produced a serum factor that could neutralize the immunosuppressive effect.*

After years of intensive investigation, the mechanisms for the induction of a primary tumor remain unresolved. However, there is an increasing body of evidence that the immune system may play an important role in retarding tumor growth. It has even been postulated that immune mechanisms were evolved primarily as a natural defense against neoplasia (*1*).

Recently, we have been studying the immune response of C57BL/6 mice to FBL-3 cells, a syngeneic leukemia induced by the Friend virus (*2*). This tumor line shows an unusual growth behavior: intraperitoneal inoculation of as few as 1×10^1 cells produces progressive tumor growth and eventually kills the hosts (progressors); in contrast, subcutaneous inoculation of up to 50×10^6 FBL-3 cells produces transient tumor growth with subsequent complete regression (regressors). The regressors then resist the intraperitoneal challenge of up to 1×10^6 FBL-3 cells. Thus, the growth of FBL-3 cells in vivo can be easily controlled by varying the route of inoculation, and it appears that this tumor might be used for studying the various factors which may determine the fate of tumor growth, and the host immune status.

A distinct feature of the growth of FBL-3 cells in the progressors and the regressors is that the intraperitoneal growth is ascitic in form and the subcutaneous growth is localized. One explanation is that factors present in the ascitic fluid promote tumor growth. We have investigated this possibility.

Ascitic fluid was collected from progressors and separated from tumor cells by centrifugation at 500*g* for 10 minutes, then at 100,000*g* for 50 minutes. The fluid was added to tissue culture medium or inoculated into mice to study its effect on the growth of FBL-3 cells in vitro and in vivo. We also inoculated mice with pleural effusion obtained from a human patient with breast carcinoma, and with benign ascites fluid from C57BL/6 mice that had been inoculated intraperitoneally with pristane (*3*).

Figure 1A shows that ascitic fluid almost doubled the growth rate of FBL-3 tissue culture cells in vitro. Figure 1 shows that subcutaneous tumor growth could be detected in the control animals given no ascitic fluid or benign ascites fluid at day 5 after inoculation, the tumor reached peak size at days 15 to 20, then regressed and became undetectable at days 30 to 40. In contrast, in the experimental animals given ascitic fluid or breast carcinoma effusion, the tumors grew much faster and many animals died of progressively growing tumors. Some tumors did eventually regress, but much

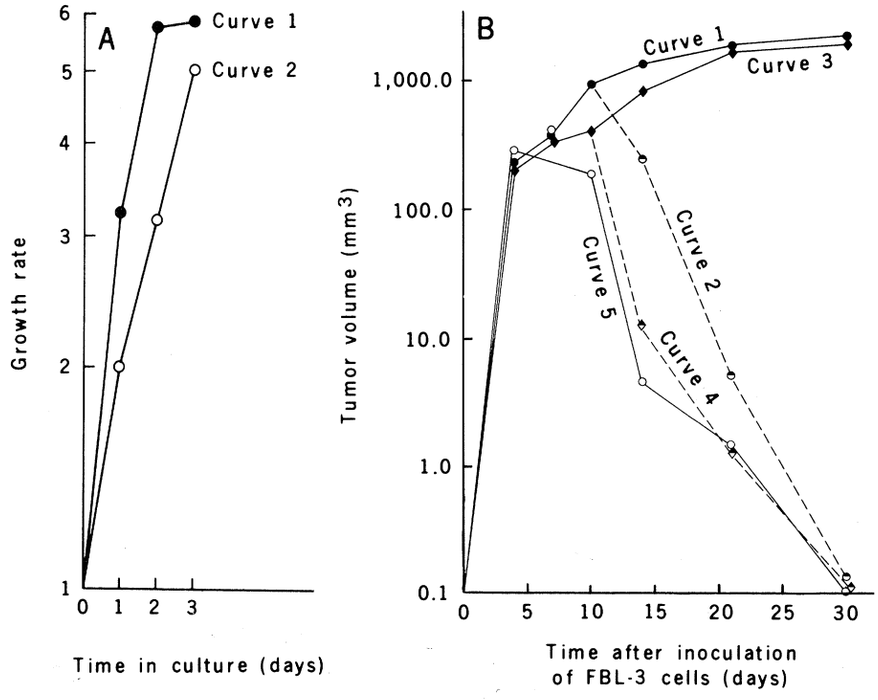

Fig. 1. Effect of FBL-3 ascitic fluid, breast carcinoma pleural effusion, and benign ascites fluid on the growth of FBL-3 cells (A) in vitro and (B) in vivo. (A) Curve 1, 10 percent ascites fluid added to FBL-3 cells grown in RPMI 1640 (Gibco) containing 20 percent fetal bovine serum. Curve 2, FBL-3 cells grown in RPMI 1640 containing 30 percent fetal bovine serum; no ascites fluid added. (B) FBL-3 cells (1.5×10^7) were inoculated subcutaneously into C57BL/6 mice on day 0; the mice then received three intraperitoneal inoculations of 0.3 ml of undiluted ascites fluid or pleural effusion at 3-day intervals (on days 0, 3, and 6). The control groups received tumor cells, and then either benign ascites fluid or no further treatment. The experiments were repeated three times or more with the same results. The incidence of development of progressively growing tumor (progressor) in both the group treated with ascites fluid and the group treated with malignant pleural effusion was significantly higher than the control group ($P \leq .01$ by the χ^2 test). Of the 18 rats that were treated with undiluted ascites, 14 became progressors (curve 1), and four became regressors (curve 2). Of the 15 mice that were treated with pleural effusion, six became progressors (curve 3), and nine became regressors (curve 4). Curve 5, control groups.

later than the controls. Pathological examination revealed that subcutaneous tumors obtained from mice without further treatment were localized and easily separated from the skin; they were surrounded by fibrous tissue with infiltration of histiocytes and lymphocytes, and there was no distant infiltration. In contrast, if the mice received FBL-3 ascitic fluid or pleural effusion from patients with breast carcinoma, those subcutaneous tumors which grew progressively showed massive local invasion to dermis and subcutaneous tissue and to the underlying abdominal muscle. Furthermore, distant infiltration was found in liver, spleen, and kidneys. Thus is was evident that a tumor growth-promoting factor was present in the malignant effusion of certain tumor-bearing hosts.

Cell-mediated immunity plays an important role in immunity to tumors in vivo. By measuring the release of ^{125}I-labeled iododeoxyuridine ([^{125}I]iododeoxyuridine release assay) we have detected the cell-mediated cytotoxic responses to FBL-3 cells in syngeneic C57BL/6 mice. These immunological reactions measured in vitro are well correlated with tumor immunity in vivo (*4*). The cytotoxic reactions were primarily directed against antigens associated with the FMR (Friend-Moloney-Rauscher) leukemia. The levels of cytotoxicity obtained with lymphocytes from the progressors were consistently lower than with lymphocytes of the regressors; and in contrast to regressors, progressors had lost their ability to mount an efficient secondary response.

In previous experiments, the cell-mediated cytotoxic response to FBL-3, as measured by the [^{125}I]iododeoxyuridine release assay, could be suppressed by treatment of the mice with FBL-3 ascitic fluid (*5*). In the present study, the breast carcinoma pleural effusion had a similar immunosuppressive effect, but the benign ascites did not (Table 1, experiment 1). Pleural effusions from two other cases of breast carcinoma and three cases of malignant lymphoma and serum from a patient with breast carcinoma also inhibited the cell-mediated cytotoxic reactions. Two pools of normal human serum (each from ten individuals) gave no inhibition.

It was also demonstrated previously that in a significant number of hosts in which the immunosuppressive effect of ascites treatment results in eventual regression of subcutaneous tumors, further treatment with ascites fluids has no effect on the secondary cytotoxic response (*5*). This suggested that a factor capable of abrogating the immunosuppressive effect of ascites had been produced. In the present experiments, therefore, serum was obtained from ascites-treated regressors and mixed with ascitic fluid to determine whether such a "counter factor" could be detected. We found that treatment with this serum abolished the immunosuppressive effect of ascites (Table 1, experiment 2, A to D), whereas normal mouse serum had no effect. In addition, serum obtained from regressors not treated with ascites (mice that received subcutaneous inoculation of FBL-3 cells) also partially inhibited the ascites effect (experiment 2E). Thus, a serum factor capable of inhibiting the immunosuppressive effect of ascites was a constant finding in animals with regressing tumors. In contrast, serum obtained from progressors (mice that received intraperitoneal inoculation of FBL-3 cells) had no effect (experiment 2F). Furthermore, if spleen cells were obtained from these same mice (experiment 2, A to D) and were further sensitized in the mixed lymphocyte tumor cell culture (*6*), an effect of the "counter factor" on the secondary cytotoxic response could be detected. The spleen cells obtained from mice that received ascitic fluid plus serum from ascites-treated regressors gave the same response as the untreated hosts (experiment 2, G and J), while spleen cells from the mice that received ascites

Table 1. Suppression of cell-mediated cytotoxic response to FBL-3 cells with malignant effusions. All experiments were repeated three to five times and were reproducible. Only representive experiments are shown. The [^{125}I]iododeoxyuridine release assay was performed in Micro Test II plates (Falcon). The target cells were FBL-3 (5×10^3 cells per well), and the ratio of effector to target cells was 500 : 1, except in experiment 2, G to J, where it was 50 : 1. All C57BL/6 mice received subcutaneous inoculation of 5×10^6 FBL-3 cells on day 0. Lymph node cells (pool of inguinal, axillary, and cervical nodes) and spleen cells were removed from these mice at 10 days after inoculation for testing. The schedule of administration of malignant effusions with or without serum was the same as in Fig. 1. In experiment 1 the mice were given three intraperitoneal inoculations of 0.3 ml of undiluted ascites fluid or effusion (protein concentration at about 30 mg/ml) at 3-day intervals after the inoculation of tumor cells. In experiment 2, the mice were given two intraperitoneal inoculations of 0.3 ml of undiluted ascites fluid (B), ascites fluid mixed with equal volumes of serum from ascites-treated regressors (ascites regressor serum) (D), normal mouse serum (C), regressor serum from mice challenged subcutaneously but not treated with ascites (E), or progressors serum from intraperitoneally challenged mice (F). For H to J, spleen cells were obtained from the mice in experiment 2, A to D, and were further sensitized in vitro with x-irradiated (10,000 rad) FBL-3 cells at a ratio of responding cells to sensitizing cells of 300 : 1. The [^{125}I]iododeoxyuridine release assay was performed 7 days after sensitization in vitro. In experiment 3, the mice were inoculated with tumor cells and then given three intraperitoneal inoculations of 0.3 ml of undiluted ascites fluid or equivalent amounts of fractions (precipitate or supernatant) obtained after precipitation of the fluid with 50 percent ammonium sulfate at 3-day intervals. The amount of supernatant G-50 used (supernatant fraction after passage through Sephadex G-50 column) was only about one-third of the original ascites fluid. The statistics were evaluated by Welch distribution *t*-test. The results were compared to net lysis (percentage) obtained with immune lymphocytes from mice without malignant effusion or serum treatment.

Treatment	Net lysis (%) obtained with			
	Lymph node	*P*	Spleen	*P*
Experiment 1				
A. None	53		45	
B. FBL-3 ascites fluid	20	< .01	13	< .01
C. Breast carcinoma effusion	33	< .01	24	< .01
D. Benign ascites fluid	56		54	
Experiment 2				
Primary cytotoxic response				
A. None	44		41	
B. FBL-3 ascites fluid	17	< .01	12	< .01
C. FBL-3 ascites fluid plus normal mouse serum	16	< .01	10	< .01
D. FBL-3 ascites fluid plus ascites-treated regressor serum	41		34	< .05
E. FBL-3 ascites fluid plus regressor serum	37	< .05	24	< .01
F. FBL-3 ascites fluid plus progressor serum	22	< .01	14	< .01
Secondary cytotoxic response				
G. None			33	
H. FBL-3 ascites fluid			11	< .01
I. FBL-3 ascites fluid plus normal mouse serum			12	< .01
J. FBL-3 ascites fluid plus ascites-treated regressor serum			31	
Experiment 3				
A. None	48		40	
B. FBL-3 ascites fluid	12	< .01	13	< .01
C. FBL-3 ascites fluid precipitate*	41	< .05	39	
D. FBL-3 ascites fluid supernatant*	18	< .01	10	< .01
E. FBL-3 ascites fluid supernatant* G-50	23	< .01	2	< .01

*After precipitation with 50 percent ammonium sulfate.

or ascites plus normal mouse serum showed marked suppression (experiment 2, H and I).

In an attempt to characterize the immunosuppressive factor, we found that: (i) It was not virus (*7*), because ultracentrifugation at 100,000*g* for 50 minutes did not reduce its effect. (ii) It did not appear to be antigen or antibody (*8*), because there was no absolute antigenic specificity; for example, human breast carcinoma effusion had a similar effect and it did not precipitate with the γ-globulin fraction. Also, ascites fluid did not produce tumor immunity (data not shown). (iii) It was probably not α-globulin (*9*), since α-globulin levels were not elevated in the ascites, as compared to normal mouse serum. After precipitation with 50 percent ammonium sulfate, most of the reactivity was found in the second fraction (supernatant fraction) (Table 1, experiment 3). This reactive fraction was excluded from Sephadex G-50, and sodium dodecyl sulfate (SDS) gel electrophoresis showed three distinct protein bands: the major one (more than 90 percent) appeared to be albumin, and there were two weaker bands with molecular weights between 40,000 and 60,000. The immunosuppressive factor might be in one or both of these two weaker bands although at this stage of purification the protein would probably not be visible on the gel.

Previous studies of immunosuppressive factors were usually conducted with systems in vitro or did not deal directly with tumor immunity (*7–9*). Our studies indicate that a humoral factor with two distinct and possibly related biological activities can be demonstrated in tumor-bearing hosts, both man and mouse: tumor growth promotion and immunosuppression. Excessive production or inability to inhibit the production of these factors is correlated with progressive tumor growth in the tumor model studied here. On the other hand, the hosts that have escaped the immunosuppressive effect produce substances that neutralize the immunosuppressive factors and such counter factors may play an important role in the control of tumor growth.

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Perilla Ketone: A Potent Lung Toxin from the Mint Plant, *Perilla frutescens* Britton

Abstract. *Perilla ketone, from the essential oil of* Perilla frutescens, *is a potent pulmonary edemagenic agent for laboratory animals and livestock. This finding would account for reported effects of the plant on grazing cattle. The use of perilla in oriental foods and medicinal preparations suggests possible hazards to human health as well.*

The discovery in our laboratory of potent lung-toxic 3-substituted furans in mold-damaged sweet potatoes (*Ipomoea batatas*) led to investigations of other naturally occurring compounds in this class. Of special interest were those produced in plants associated with foods of animals and man. The close chemical similarity of perilla ketone to the lung-toxic ipomeanols from sweet potatoes indicated that the former compound would likely exhibit similar toxic properties. We have found that both perilla ketone and crude extracts from the plant source *Perilla frutescens* show potent lung toxicity for experimental animals. This fact should serve to explain reported outbreaks of atypical pulmonary emphysema in cattle that grazed the plant and points to possible health hazards for humans using the plant or its essential oil in oriental food and medicinal preparations.

Perilla frutescens, known in the United States as "purple mint plant," "beefsteak plant," "perilla mint," and "perilla," is an import from Asia that was, apparently, a garden escapee and became widely distributed in midwestern, eastern, and southern United States (*1*). *Perilla frutescens* and related species are also found in certain European countries and have been used extensively in plant biochemistry and physiology studies in the United States (*2*), the Soviet Union (*3*), and elsewhere (*4*).

In Japan, species of *Perilla* and related plants grow wild, and several varieties are cultivated for various uses (*5*). The anthocyanins of highly tinted perilla leaves are extracted and used as coloring agents for green plums. Intact leaves may be used as condiments or flavoring agents in a variety of human foods and are often included in preparations of tempura deep-fried with batter. Perilla seeds are available as bird feed at pet shops in Japan and are used to flavor various foods, including pickled ginger. Volatile (essential) oil from the leaves and other parts of the plant (particularly *P. frutescens*, var. *crispa*, *forma viride*) is known in Japan as "Ao-shiso"; in 1958–59, approximately 5 to 7 metric tons of perilla oil were produced in Japan (*5*). "Ohara shiso" (variety *crispa* Decaisne) is reported to be the source of the Chinese drug called "soyo" that reportedly has several therapeutic applications in humans (*6*).

Perilla seed oil, which is commercially available, has been evaluated as a drying oil for paints and lacquers (*7*). Perilla seed oil cake, or meal, has been used both as a fertilizer and a high protein animal feed (*8*).

The volatile oils of different species and varieties of *Perilla* vary considerably as to main constituents. In some varieties of *P. frutescens*, 1-perillaldehyde is the principal component imparting a pungent aroma to the oil (*9*). The α-*syn*-oxime of 1-perillaldehyde (*10*), called perillartine, is a potent sweetening agent often added to Japanese tobacco. Other varieties may contain perilla ketone (**1**) (*11*), egomaketone (**2**) (*12*), and isoegomaketone (**3**, Fig. 1) (*13*) as predominant compounds in the plant essential oil. Chemical structures of these 3-substi-