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# *In Vivo* Antitumor Activity of the Bitter Melon (*Momordica charantia*)[1]

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## ABSTRACT

The *in vivo* antitumor activity of a crude extract from the bitter melon (*Momordica charantia*) was determined. The extract inhibited tumor formation in CBA/H mice which had been given i.p. injections of $1.0 \times 10^5$ CBA/DI tumor cells (77% of the untreated mice with tumors *versus* 33% of the treated mice with tumors after 6 weeks). The extract also inhibited tumor formation in DBA/2 mice which had been given i.p. injections of either $1 \times 10^5$ P388 tumor cells (0% of untreated mice survived after 30 days *versus* 40% survival of the treated mice) or $1 \times 10^5$ L1210 tumor cells (0% survival of untreated mice *versus* 100% of treated mice after 30 days). The *in vivo* antitumor effect required both the prior exposure of tumor cells to the extract (2 hr) *in vitro* and i.p., biweekly injections of the extract into the mice. The optimum dose for tumor inhibition (8 μg protein, biweekly, i.p.) was not toxic to mice for at least 45 days of treatment. This same treatment caused a marked enhancement of C3H mouse thymic cell response to concanavalin A *in vitro*. When compared to the untreated control mice, the bitter melon-injected animals exhibited a 4-fold-higher incorporation of tritiated thymidine into trichloroacetic acid-precipitable material after 48 hr of exposure to 50 μg of concanavalin A. Nylon wool-purified spleen cells from these same bitter melon-treated mice exhibited an enhanced mixed lymphocyte reaction when exposed to irradiated P388 stimulator cells (186% of the untreated control mice). These data indicate that *in vivo* enhancement of immune functions may contribute to the antitumor effects of the bitter melon extract.

## INTRODUCTION

The bitter melon plant (*Momordica charantia*) contains several seed lectins which, while not highly toxic to animals *in situ*, will inhibit protein synthesis *in vitro* (13). Lin *et al.* (13) reported that 2 such lectins with molecular weights corresponding to 32,000 and 24,000 could be purified. These lectins inhibited Ehrlich ascites cell growth at relatively high concentrations (100 μg/ml or greater). The 2 purified lectins are called momordin ($M_r$ 24,000 protein) and agglutinin ($M_r$ 32,000 protein). The former inhibited protein synthesis (12–14). Barbieri *et al.* (1) reported that an additional $M_r$ 115,000 seed lectin inhibited protein synthesis in a rabbit reticulocyte lysate system.

These lectins cause no apparent harm to animals when injected i.p. at 1 mg/100 g body weight (1). It is hypothesized that, like modeccin and similar toxins, these lectins cannot enter cells.

We (22, 26) and others (3) have observed similar activities in an aqueous extract from the bitter melon fruit. In these cases, however, cytostatic and cytotoxic efffects were apparent on intact cells in culture. Vesely *et al.* (27) and Claflin *et al.* (3) reported that an aqueous extract of the ripe fruit contained both a guanylate cyclase enzyme inhibitor and a cytostatic factor which blocked rat splenic lymphocytes at the $G_2$-M phase of the cell cycle (3). We have also found that similar extracts prevent both Con A[3]-stimulated thymidine incorporation into human peripheral blood lymphocyte DNA and a subsequent induction of a specific cyclic AMP phosphodiesterase (25).

This crude extract acts as a competitive inhibitor of guanylate cyclase activity *in vitro* and lowers cellular cyclic GMP levels *in vivo* (23). Inhibition is greater for the enzyme activity from leukemic lymphocytes when compared to normal human lymphocytes (245 μg/ml for normal human lymphocytes *versus* 170 μg/ml for leukemic human lymphocytes) (23).

The most intriguing observation is the marked preferential cytotoxic effect of the extract for human leukemia cells (22). The $LD_{50}$ is greater than 5000 μg/ml for normal human peripheral blood lymphocytes *versus* 300 to 400 μg/ml for lymphocytes from patients with chronic or acute leukemia (8, 22, 23).

We have purified recently several of these cytostatic and cytotoxic factors (24). One such factor has a molecular weight corresponding to 50,000 to 70,000. This protein retains both guanylate cyclase-inhibitory activity and a preferential cytostatic effect on leukemic lymphocytes (22).

Our earlier observation that the crude extract contained multiple factors (26) has led recently to the purification of several additional components (24). One of these components also exhibits antiviral activity against vesicular stomatitis virus.

Finally, the observation that the crude extract from the bitter melon fruit reduced the occurrence of rat prostate adenocarcinoma *in vivo* (5) led us to attempt similar studies with several murine lymphoma systems. Although of a preliminary nature, these studies indicate clearly that the crude extract inhibits *in vivo* lymphoma formation in mice. Furthermore, this same extract appears to enhance the immune functions of these animals.

## MATERIALS AND METHODS

The DBA/2 and C3H/HeN ($MTV^-$) mice (6 weeks to 2 months old, female) were obtained from the NIH. CBA/H mice were propagated at the University of California (Los Angeles). Con A was from Sigma Chemical Co. (St. Louis, Mo.).

Bitter melons (*M. charantia*, also called balsam pear) are grown locally and prepared initially as described (22). The whole ripe fruits (10 to 20 lb) are homogenized in cold PBS, filtered through cheesecloth, and then centrifuged at 16,300 × *g* (Sorvall GSA) for 20 min. The resulting supernatant is precipitated to 50% saturated ammonium sulfate, and the resulting pellet (from the above centrifugation step) is taken up in PBS and dialyzed against PBS overnight to remove residual ammonium sulfate. All procedures are conducted at 4° unless otherwise stated. This crude aqueous extract is stored at −70° in 50-ml batches and used as

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[3] The abbreviations used are: Con A, concanavalin A; cyclic AMP, cyclic adenosine 3′:5′-monophosphate; cyclic GMP, cyclic guanosine 3′:5′-monophosphate; $LD_{50}$, dose lethal to 50% of the lymphocytes; $MTV^-$, mammary tumor virus negative; PBS, phosphate-buffered saline [NaCl (8.00 g/liter):$KH_2PO_4$ (0.20 g/liter):$Na_2HOP_4$ (1.70 g/liter), pH 7.2]; MLR, mixed-lymphocyte reaction.

the source of crude bitter melon preparation. This extract is stable for up to 1 year at −70°.

The *in vitro* dose-response curves are determined in 24-well microtiter plates (Costar, Cambridge, Mass.). Each well contained $1 \times 10^5$ cells, bitter melon extract or PBS, and medium to 1-ml final volume. The plates are incubated for 24 hr at 37° in an atmosphere of 95% air:5% $CO_2$. Cell counts and viability are determined by hemocytometer counting and trypan blue dye exclusion, respectively.

Toxicity studies were conducted on 2-month-old C3H/HeN ($MTV^-$) mice (male and female). Filter-sterilized (0.2-μm Acrodiscs; Gelman Instrument Co., Ann Arbor, Mich.) crude bitter melon extract was injected either i.p. or s.c. at doses of 8, 11, 16, 27, 31, and 50 μg protein per ml. Control animals received similar injections of sterile PBS. Animals were weighed and examined for gross pathological alterations.

*In vivo* antilymphoma activity was determined using 2-month-old CBA/H mice. The animals were given injections of a CBA/DI T-cell lymphoma line established from a thymus of a CBA/H mouse with lymphomas induced by Gross murine leukemia virus. When injected i.p. at $10^4$ cells/mouse, the cells cause lymphomas and death within 30 days postinoculation in most of the mice.

All cell lines were maintained in Roswell Park Memorial Institute Tissue Culture Medium 1640 (Grand Island Biological Co., Grand Island, N. Y.), containing 10% calf serum (Dutchland Chemicals, Denver, Pa.), L-glutamine, and a penicillin:streptomycin mixture. These suspension cultures are grown in an atmosphere of 5% $CO_2$:95% air.

Antilymphoma activity was measured in 6-week-old female DBA/2 mice given injections i.p. of either $1 \times 10^5$ P388 cells or $1 \times 10^5$ L1210 cells (both from American Type Culture Collection, Rockville, Md.).

Polyclonal lectin activation was measured in 6-week-old C3H/HeN ($MTV^-$) mice which were given injections i.p. with 8 μg of protein of the bitter melon extract, biweekly, for 30 days. Control animals received PBS injections. Thymic lymphocytes were removed under sterile conditions, depleted of adherent cells, and purified on glass wool columns (11). Cells were cultured in 24-well microtiter plates at $2 \times 10^7$ cells/ml as described above. Cells were harvested at various times after exposure to Con A.

For the final 3 hr of incubation, cultures were pulsed with 2 μCi each of tritiated thymidine ([*methyl*-$^3$H]thymidine; 60 to 90 Ci/mmol; ICN Chemical Radioisotopes Div., Irvine, Calif.) and then harvested onto Whatman GF/A filters (Whatman, Inc., Clifton, N. J.). Filters are sequentially washed with PBS (3 times), 10% trichloroacetic acid, and 70% ethanol and then counted by liquid scintillation counting.

MLRs are measured in the same 6-week-old C3H/HeN ($MTV^-$) mice which were used for polyclonal lectin activation studies. Mouse spleen cells are harvested, purified under sterile conditions, and cultured as described above at $1.5 \times 10^6$ cells/ml for 3 days. Cells are stimulated with various numbers of irradiated P388 stimulator cells (Model 40 γ-cell small-animal irradiator; Atomic Energy of Canada, Ltd.; 2000 rads). For the last 8 hr of incubation, cultures are pulsed with 2 μCi of tritiated thymidine and then processed on GF/A filters as described above.

## RESULTS

The $LD_{50}$ doses for the crude bitter melon extract were determined both i.p. and s.c. The $LD_{50}$ was 27 μg of protein per ml when injected s.c. and 16 μg of protein per ml when injected i.p. When mice were given injections of the $LD_{50}$ dose, death occurred within 3 to 5 days. When repeated lower doses were given (11 and 16 μg of protein per ml; Table 1), animal weight gain was stunted. However, animals appeared alert and fed normally. Weight returned to normal following termination of the injections (data not shown). Two mice from each group were sacrificed after 30 days and examined. No gross lesions were reported in any organ. However, a mild hepatic hyperplasia, some depletion of body fat, and a lower WBC were found, when compared to PBS-treated control animals (data not shown).

Table 1

*Effects of s.c. and i.p. injections of crude bitter melon extract on growth of C3H mice*

The 2-month-old C3H/HeN ($MTV^-$) mice (2/group) were given injections i.p. every 3 days with bitter melon extract beginning on Day 1. Protein concentrations of inocula were determined by the Coomassie dye procedure (2). Results are the average of 2 animals.

| Route of administration | Dose (μg protein/ml) | Animal wt (g) at the following days of treatment | | |
|---|---|---|---|---|
| | | 1 | 30 | 45 |
| s.c. | 0 (control) | 19.0 | 25.5 | 28.0 |
| | 8 | 17.5 | 23.0 | 28.5 |
| | 11 | 18.2 | 23.5 | 27.0 |
| | 16 | 17.0 | 18.0 | 20.5 |
| i.p. | 0 (control) | 17.6 | 26.0 | 28.0 |
| | 8 | 18.0 | 23.5 | 25.7 |
| | 11 | 19.2 | 20.0 | 21.2 |
| | 16[a] | 17.6 | | |

[a] Animals that were given injections i.p. of 16 μg of protein per ml died.

Table 2

*In vitro dose-response of tissue culture cells to crude bitter melon extract*

| Bitter melon[a] (μg/ml) | CBA/DI cells (% viable)[b] |
|---|---|
| 10 | 85 |
| 20 | 75 |
| 50 | 50 |
| 100 | 30 |
| 150 | 20 |
| 200 | 10 |
| 500 | 0 |

[a] The crude bitter melon extract was added in PBS (filter sterilized) to the microtiter wells at the doses indicated (μg protein per ml). Protein concentration was determined by the Coomassie dye procedure (2).

[b] Percentage of viability was determined by trypan blue dye exclusion after 24 hr of exposure to the bitter melon. The cells were cultured as described in "Materials and Methods." These results are the average of duplicate experiments.

As indicated in Table 2, the crude extract is cytotoxic to the CBA/DI cells in culture. This toxicity is dose dependent and requires 24 hr before it becomes apparent. However, cells exposed to the crude extract for 30 min, washed, and then incubated for 24 hr will also exhibit a decreased viability (22). Thus, the constant presence of the crude extract is not required.

Although the crude extract was cytotoxic to CBA/DI cells *in vitro* (Table 2) after 24 hr of incubation, this treatment alone will not reduce the incidence of tumors (Table 3). No reduction in lymphoma formation was found, even when $10^5$ CBA/DI cells were incubated with 100 μg of bitter melon. It appears that injection of bitter melon directly into the mice is also necessary for antilymphoma activity. Thus, the antitumor activity of this extract may not be entirely a result of a direct effect on the CBA/DI cells.

As indicated in Table 4, the injection of bitter melon i.p. into the mice does not cause an overall decrease in tumor incidence. However, a slight delay in the death of the animals is observed (5 of 6 mice by 53 days in the control groups *versus* 7 of 8 mice by 75 days in the group receiving injections beginning on Day 3).

The greatest antilymphoma effects were noted both when the CBA/DI cells were pretreated with the bitter melon extract and when animals were given injections biweekly (Table 5).

If animals were given 8 μg of bitter melon extract biweekly following the injection of pretreated lymphoma cells, the incidence of lymphoma formation was dramatically decreased (controls, 77% with lymphomas *versus* 30 to 33% of treated group).

Table 3

*Effect of in vitro pretreatment of CBA/DI cells with crude bitter melon extract on subsequent lymphoma formation in mice*

| Dose of bitter melon (μg protein/ml medium) | Animals with tumors[a] (no. dead/no. in group) | % dead at 60 days |
|---|---|---|
| 10 | 9/10 (33–45)[b] | 90 |
| 20 | 10/10 (31–38) | 100 |
| 50 | 9/10 (31–38) | 90 |
| 100 | 8/10 (31–41) | 80 |
| Control | 6/7 (31–38) | 85 |

[a] CBA/DI cells ($1 \times 10^5$) were incubated with varying doses of crude bitter melon extract for 2 hr at 37°. These cells were then washed once in PBS and inoculated, i.p., into mice. Cells were greater than 95% viable at the time of inoculation. No crude bitter melon was injected into the mice directly. Control cells were incubated in medium for 2 hr prior to i.p. injection.

[b] Numbers in parentheses, range of days after cells were given.

Table 4

*Effects of multiple i.p. administration of the crude bitter melon extract on the lymphoma incidence in CBA/DI-inoculated mice*

| Dose of bitter melon (μg protein/ mouse) | Schedule[a] | Animals/ study | Animals dead of lymphoma | % with tumors in 60 days |
|---|---|---|---|---|
| 0 (control)[b] | No bitter melon treatment | 6 | 5 (24–53)[c] | 84 |
| 8 | Biweekly 8-μg injection: 3 days after cells given | 8 | 7 (34–75) | 87 |
| 8 | 8 μg biweekly: begin 6 days after cells given | 9 | 5 (39–93) | 55 |
| 8 | 8 μg biweekly: begin 9 days after cells given | 10 | 7 (24–76) | 70 |

[a] The crude bitter melon factor was given i.p. beginning 3, 6, or 9 days after the inoculation with the CBA/DI cells for 6 weeks.

[b] All CBA/H mice (2 months old) were inoculated i.p. with $1 \times 10^4$ CBA/DI cells per mouse at Day 1. Each animal was checked for tumor formation every other day. Cells were viable at the time of inoculation as judged by trypan blue dye exclusion.

[c] Numbers in parentheses, range of days after cells were given.

Table 5

*Effect of multiple i.p. administration of the crude bitter melon extract on the lymphoma incidence of the mice after inoculation with bitter melon-treated CBA/DI lymphoma cells*

| Dose of bitter melon (μg protein/ml *in vitro*) | Schedule[a] of *in vivo* injections | Animals/ study | Animals dead of lymphoma | % with tumors in 60 days |
|---|---|---|---|---|
| 8[b] | Cells treated *in vitro*; no bitter melon injections *in vivo* | 18 | 14 (24–53)[c] | 77 |
| 8 | 8 μg; biweekly; begin 3 days after cells given; for 6 wk, total | 9 | 3 (62–73) | 33 |
| 8 | 8 μg; biweekly; begin 3 days after cells given for 3 mos., total | 10 | 3 (75–123) | 30 |

[a] The crude bitter melon factor was given i.p. in biweekly doses of 8 μg of protein per mouse, beginning at 3 days postinoculation of CBA/DI cells. The CBA/DI cells were pretreated as described in Table 3 with 8 μg of bitter melon per ml.

[b] All animals were inoculated on Day 1, i.p., with $1 \times 10^5$ bitter melon-treated CBA/DI cells. Experiments were conducted as described in "Materials and Methods."

[c] Numbers in parentheses, range of days after cells were given.

Furthermore, after 73 days of bitter melon treatment, these animals did not get lymphomas if treatment was stopped (observed for up to 6 months). At this point, we assume that all of the CBA/DI tumor cells have been killed.

Table 6 illustrates that the bitter melon extract also prevents tumor formation in DBA/2 mice given injections of either P388 or L1210 tumor cells. The tumor inhibition observed in these animals also required exposure of both animals and tumor cells to the bitter melon extract.

Since the antilymphoma effect was only apparent when both tumor cells and host animals were exposed to the bitter melon extract, we wished to determine if *in vivo* treatment with this extract enhanced the immune responses of the mice.

Because the ability to reject a tumor *in vivo* is considered partially a result of a T-cell response against the tumor, we measured 2 T-cell responses, the ability of T-cells to proliferate when exposed to Con A and that when exposed to foreign tumor cells.

Chart 1 illustrates the results of exposing nylon wool-purified mouse thymic T-cells to Con A. These cells were taken from C3H/HeN ($MTV^-$) mice which had been given injections i.p. with either PBS or bitter melon extract for 30 days. After exposure of these cells *in vitro* to Con A (50 μg/ml), the cells from the bitter melon-treated animals incorporated more tritiated thymidine at an earlier time period than did those T-cells obtained from PBS-treated mice. Since the bitter melon extract was not added directly to the *in vitro* incubation, we assume that these differences in response to Con A must be a result of *in vivo* exposure to the extract. This enhanced response to Con A was observed as (*a*) greater incorporation of tritiated thymidine into cells, (*b*) earlier time of peak responsiveness, and (*c*) responsiveness at a lower concentration of Con A (data not shown). *In vivo* treatment is not directly mitogenic to the mouse thymic cells. Rather, another signal (Con A, in this case) is needed. We are uncertain as to the mechanism by which the extract primes these cells *in vivo*. Small doses of the extract, added *in vivo* to normal thymoctyes, with Con A, have no effect on subsequent mitogen-induced proliferation. However, at higher doses (around 1 μg of protein), the extract inhibits Con A-induced proliferation of normal mouse thymocytes. No effect is noted on MLR-induced proliferation of normal mouse spleen cells (data not shown).

Table 7 illustrates the results of an MLR using nylon wool-purified spleen cells obtained from the same animals as described in Chart 1. The cells isolated from the bitter melon-injected animals exhibited a greater MLR response to the irradiated P388 stimulator cells than did those cells purified from the PBS-injected control mice (about 180% of the control group). This enhanced reaction was seen when varying the effector:stimulator ratio from 1:1 to 1:100. The optimum ratio was the same for each group, however. Since the bitter melon extract was not added to the *in*

Table 6

*Effect of multiple i.p. administration of the bitter melon extract on tumor incidence in DBA/2 mice after injection with pretreated L1210 or P388 tumor cells*

| Treatment | Animals/group | Survival time (% alive after 30 days) |
|---|---|---|
| L1210 | | |
| Control group[a] | 5 | 0 |
| Bitter melon-treated group | 5 | 100 |
| P388 | | |
| Control group | 5 | 0 |
| Bitter melon-treated group | 5 | 40 |

[a] DBA/2 mice (females, 6 weeks old) were each given an injection i.p. of $1 \times 10^5$ P388 or L1210 cells. Cells were pretreated, prior to injection, with either PBS (control group) or with 8 μg of protein of the bitter melon extract (bitter melon-treated group) for 3 hr at 37°. These treated cells were washed 3 times in sterile PBS and injected into the mice. The animals in the bitter melon-treated group were given injections biweekly with 8 μg of bitter melon for 30 days.

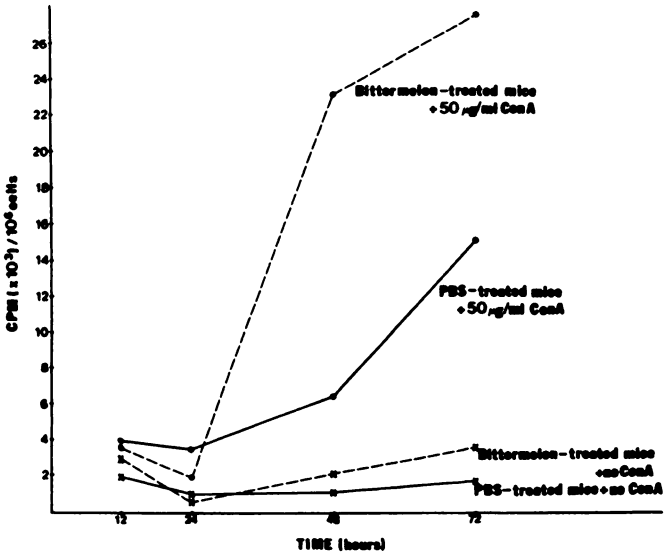


Chart 1. Effects of *in vivo* treatment of C3H/HeN (MTV⁻) mice with bitter melon extract on thymic cell polyclonal lectin activation. Mice (5/group) were given injections i.p. of 8 μg protein of crude bitter melon extract in sterile PBS (bitter melon-treated mice) or of 100 μl of PBS (PBS-treated mice) twice weekly for 30 days. Two days following the final injection schedule, thymic cells were harvested and added to microtiter wells at $2 \times 10^7$ cells/ml in 2 ml of Roswell Park Memorial Institute Tissue Culture Medium 1640 plus 10% fetal calf serum. Each sample was pulsed for 3 hr with 2 μCi of tritiated thymidine and then harvested on Whatman GF/A filters at the indicated time period. Results are averages of duplicate experiments on the pooled cells from 5 mice.

Table 7

*Effects of bitter melon treatment in vivo on mouse MLR response*

| Sample[a] | P388 stimulator cells (×10⁶) | Response (cpm tritiated thymidine) | % of control |
|---|---|---|---|
| PBS-treated mice | 1 | 11,226 | |
| | 10 | 12,279 | |
| | 25 | 15,835 | |
| | 50 | 45,809 | |
| | 100 | 45,310 | |
| | 200 | 43,834 | |
| Bitter melon-treated mice | 1 | 19,554 | 174 |
| | 10 | 20,672 | 168 |
| | 25 | 29,518 | 186 |
| | 50 | 85,822 | 187 |
| | 100 | 55,114 | 121 |
| | 200 | 43,640 | 99 |
| Irradiated P388 cells, alone (200 × 10⁶) | | 548 | |
| Medium, alone | | 422 | |
| Spleen cells, alone (1.5 × 10⁶) | | 1,375 | |

[a] Mouse spleen cells were purified (11) from C3H/HeN (MTV⁻) mice (2 months old) which had been given injections i.p. biweekly of PBS or 8 μg of protein of the bitter melon extract for 30 days. Cells were harvested 2 days after the final injection of bitter melon. Spleen cells ($1.5 \times 10^6$) were incubated in 2 ml of medium for 3 days at 37° in 95% air:5% $CO_2$. Varying numbers of irradiated P388 cells were added as stimulators. Cultures were pulsed for 8 hr with 2 μCi of tritiated thymidine and processed for liquid scintillation counting on GF/A filters as described. These are the results of duplicate experiments on cells pooled from 3 animals in each group.

*vitro* incubation, we assume that these differences are due to *in vivo* effects of the extract on the mouse spleen cells.

## DISCUSSION

There is a tremendous legacy of folklore involving the use of plant preparations in medicine. Hartwell (8) has reported that over 3000 species have been used in cancer treatment alone. Among those utilized most recently are maytansine and vincristine, both of plant origin (21, 28).

Until recently, very little was known about specific anticancer proteins from plants. Although plant proteins which are toxic to animal cells are widely distributed among different species (7), their mechanisms of action are not known in detail.

However, considerable progress has been made on the mechanism of action of plant toxins, usually of seed origin. Among these toxins, abrin and ricin have been studied most extensively (12, 14). These seed toxins are from *Abrus precatorius* and from *Ricinus communis*, respectively. While both abrin and ricin are extremely toxic to normal cells, they have been reported to be more toxic towards malignant cells.

The toxicity, *in vivo*, appears to depend on the presence of both toxin subunits; the B chain is needed for cellular binding, while the A chain shuts off protein synthesis (16). The latter acts enzymatically by preventing the ribosomal-linked GTP hydrolysis catalyzed by Elongation Factor 2 at the 60S ribosomal subunit (20).

Initially, these seed toxins appeared to be too toxic for use as anticancer agents. Recently, however, through the specific coupling of abrin or ricin to monoclonal antibodies directed against tumor cell surface antigens, a more "selective delivery" has been achieved (9). For example, Thy 1.1-specific monoclonal antibodies covalently linked to ricin will selectively kill only Thy 1.1-positive cells in culture (9).

Other plant seed toxins appear to act by the same mechanism (7). However, some do not bind to cells and therefore only inhibit protein synthesis in cell-free systems (7). Among this group are modeccin (6, 19) and momordica lectins (1).

Recently, plant lectins have been found to inhibit tumor formation *in vivo* (4). Although a direct effect is observed in the tumor cell, enhancement of host-cell immune functions is also observed. *Griffonia simplicifolia* ganglioside I lectin, for example, prevents *in vivo* formation of Ehrlich ascites tumors in mice when injected i.p. (4). It appears that direct interaction of tumor cells with the lectin is required. Pretreatment of Ehrlich ascites cells *in vitro* for 1 hr prevented subsequent tumor formation by these same cells when injected into the animal.

However, the lectin-mediated prevention of tumor formation also involved activation of peritoneal macrophages which mediate tumor cell lysis (15). *In vitro* incubation of macrophages caused these cells to develop a ganglioside I-binding cell surface glycoprotein. Appearance of this glycoprotein correlated with the appearance of activated macrophages. It has been proposed that treatment of the tumor cells and macrophages facilitates recognition by both cell types.

Although this is an attractive hypothesis, it does not fully explain the effects which we have observed in our system.

We have observed a markedly enhanced reaction of mouse thymic and spleen cells to Con A and in a MLR, respectively. Since both the tumor cells and bitter melon extract are administered i.p., this implies that there may be a generalized enhancement of host animal immune functions after treatment with the extract.

Enhancement of immune functions could occur through the action of lectins which may be present in the crude bitter melon extract. However, in our system, stimulation of immune functions does not appear to be sufficient for tumor inhibition. Rather, interaction of some component in the bitter melon extract directly

with the tumor cells is also required. At this point, we cannot determine if an immune enhancement contributes to the antitumor effect of the bitter melon extract. Further studies showing a reduction of antitumor activity of the extract in immune-deficient mice would answer this question.

We feel that there may be several components in this extract which are acting via different mechanisms.

We have purified several antileukemia factors previously from this extract (24, 26). One such factor acts as a competitive inhibitor of human lymphocytic guanylate cyclase *in vitro* and lowers cellular cyclic GMP levels of human leukemia cell lines in culture (23).

The 50%-inhibitory dose for purified soluble guanylate cyclase inhibition by the bitter melon factor is around 7 $\mu$g of protein for the leukemic cell enzyme and around 100 $\mu$g of protein for the enzyme from normal human lymphocytes (23).

Another intriguing observation is the marked preferential cytotoxic (at higher doses) or cytostatic (at lower doses) effect of the factor for human leukemia cells (22, 26). This preferential effect could be due to the presence of the more sensitive guanylate cyclase found in the leukemia cells (22, 26).

Since cyclic GMP is elevated in these leukemic cells (22, 26), the inhibition of guanylate cyclase could serve to return the cyclic AMP:cyclic GMP ratio to normal, resulting in a decreased rate of cell proliferation and, perhaps, cell death.

It has been demonstrated recently that *in vitro* or *in vivo* manipulation of human lymphocytic and monocytic cyclic AMP or cyclic GMP levels alters dramatically both cell growth and specific cell differentiation of both normal and neoplastic cells (10, 11, 17, 18).

Further purification of these factors from the bitter melon extract should determine which are necessary for optimum *in vivo* antitumor activity and what their mechanisms of action are.

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