Modulation of macrophage tumoricidal capability by polyene antibiotics: Support for membrane lipid as a regulatory determinant of macrophage function

(macrophage differentiation/amphotericin B/ionophores)

HAROLD A. CHAPMAN, JR. AND JOHN B. HIBBS, JR.

Veterans Administration Hospital and Department of Medicine, Division of Infectious Diseases, University of Utah Medical Center, Salt Lake City, Utah 84148

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ABSTRACT We have examined the effects of the sterol-binding polyene antibiotics on macrophage tumoricidal capability. Incubation for 2 hr of activated macrophages from bacillus Calmette-Guerin-infected mice with amphotericin B at 0.5-2 μg/ml or amphotericin B methyl ester at 0.5-10 μg/ml enhanced the capability of activated macrophages to kill 3T12 cells. These polyenes did not make normal or stimulated macrophages tumoricidal. Experiments with the ionophores gramicidin, alamethicin, nigericin, and valinomycin indicate that the ionophoretic properties of amphotericin B may not account for its enhancing effect on macrophage tumoricidal potential. Two polyenes with a smaller ring structure, filipin and pimaricin, were also ineffective suggesting that stereospecific modifications in membrane lipid organization underlie the enhancing effect of amphotericin B. The results suggest that the clinical efficacy of amphotericin B in promoting resistance to fungal disease and possibly to neoplasia may operate in part through potentiation of macrophage effector functions.

Recent observations from this laboratory suggest that tumor cell killing by activated macrophages is highly dependent on the local environment in which macrophages and tumor cells coexist (1). Activated macrophages are tumoricidal in vitro when cultured in medium supplemented with fetal bovine serum, but these same macrophages generally do not kill tumor cells or do so only variably when cultured in medium supplemented with adult sera. We have termed such macrophages nontumoricidal activated macrophages. We have described several factors in normal sera, e.g., low density lipoprotein and a separate lower-molecular-weight serum factor, that regulate macrophage tumoricidal capability. Evidence was also presented that the modulation of macrophage tumoricidal potential by such serum factors may in part be dependent on cholesterol flux within macrophage plasma membranes (2). Based on these observations we have examined the effects of the sterol-binding polyene antibiotics on macrophage tumoricidal capability.

METHODS AND MATERIALS

Macrophage Monolayers. Adherent cell monolayers of normal, peptone-stimulated, or bacillus Calmette-Guerin (BCG)-activated peritoneal macrophages from C3H/HeJ (Jackson Laboratories), C3H/HeN, or C57bl/6 (Frederick Cancer Research Center Animal Facility) female mice were prepared as described (2, 3).

Macrophage Activating Factor (MAF) Preparation. Lymphokine-rich supernatants with MAF activity were supplied by Brice Weinberg. The supernatants were prepared by NaI4 stimulation of peritoneal cells by a modification of the method of Bressler et al. (3, 4). Although it is recognized that these supernatants may contain factors in addition to MAF, for this report the preparation is termed “MAF.”

Cytotoxicity Assay. In vitro macrophage-mediated cytotoxicity toward tumorigenic 3T12 target cells was assayed by morphologic observation. After culture for 60 hr at 37° in humidified 95% air-5% CO2, the 3T12 cells were fixed in methanol and stained with Giemsa. When tumor cells were stained immediately after attachment to a macrophage-free chamber surface, 33-37 cells were seen per ×300 microscopic field. By 60 hr in culture, the chambers containing 3T12 cells alone had a multilayer of cells. Inhibition of 3T12 cell growth among macrophage monolayers was graded as follows: no effect, multilayer of 3T12 target cells; slight cytotoxicity, patchy areas of confluent target cell growth among macrophages; cytostasis, 30-40 3T12 cells/×300 field; weak cytotoxic effect, 5-30 3T12 cells/×300 field; and marked cytotoxic effect, <5 3T12 cells per ×300 field.

Reagents. Reagents were of the highest commercial grade available. Amphotericin B (Fungizone) and nystatin (Squibb) were obtained through the local pharmacy; amphotericin B (powder), amphotericin B methyl ester, and gramicidin were supplied by Squibb Research Laboratories; pimaricin was a gift from Mycofarm–Delft Laboratories in Delft, Holland, and from Martin Kunstmann of Lederle Laboratories; nigericin was supplied by R. L. Hamill of Lilly Research Laboratories; filipin and alamethicin were obtained from George Whitfield of the Upjohn Co. All of the sera, polyene antibiotics, and ionophores used in these experiments were endotoxin-negative when tested by the limulus amebocyte lysate assay (5).

RESULTS

Modulation of Macrophage Tumoricidal Potential by Amphotericin B and Amphotericin B Methyl Ester. In a preliminary series of experiments the toxic concentrations of amphotericin B and amphotericin B methyl ester for peritoneal macrophages were determined. By gross morphology and trypan blue exclusion, discernible toxicity to BCG-activated macrophages was observed with amphotericin B at 2 μg/ml after a 60-hr exposure. At concentrations >4 μg/ml there was rapid lysis of the BCG-activated macrophage monolayers. It had been previously found that BCG-activated mouse macrophages were more susceptible to lysis by amphotericin B than were normal or stimulated mouse peritoneal macrophages (unpublished data). Toxic changes with normal or stimulated macrophages were only evident with concentrations of amphotericin B > 8 μg/ml.

Fig. 1 is a photograph of a typical experiment that examines...
4350 Cell microtiter Falcon culture medium tericin obtained 60 The stimulated stimulated, normal, 1.5 jtg/ml rophages. incubated with (rows nor tion-3 growth were macrophages. These with tumoricidal macrophagesg (row B). concentrations of 3T12 target inhibition complete as nontumoricidal of microscopy harvested macrophages of was tumoricidal of BCG-activated macrophages. The cultures were incubated for 0.5-2i g/ml (row D), 1.0, g/ml (row E), 0.25-2 mg/ml (row F), or 2.0 mg/ml (row G). In this experiment normal (N), stimulated (S), and nontumoral BCG-activated macrophages (A) were obtained from C3H/Hes mice. The cultures were incubated for 60 hr in culture medium supplemented with 10% adult bovine serum. The 3T12 cells (T) stain darkly and macrophages (N, S, or A) are not discernibly stained.

FIG. 1. The figure is a low power photograph of a giemsa-stained Falcon microtiter plate in which each circle represents an individual culture. The macrophage monolayers were preincubated for 2 hr with tissue culture medium (row A), serum fraction-3 (row B), or amphotericin B at 0.25 μg/ml (row C), 0.5 μg/ml (row D), 1.0 μg/ml (row E), 1.5 μg/ml (row F), or 2.0 μg/ml (row G). In this experiment normal (N), stimulated (S), and nontumoral BCG-activated macrophages (A) were obtained from C3H/Hes mice. The cultures were incubated for 60 hr in culture medium supplemented with 10% adult bovine serum. The 3T12 cells (T) stain darkly and macrophages (N, S, or A) are not discernibly stained.

The effect of amphotericin B on the tumoricidal capability of normal, stimulated, and nontumoral BCG-activated macrophages. In this experiment the macrophage monolayers were incubated with amphotericin B for 2 hr at 37°C and then the monolayers were washed twice with phosphate buffered saline before the 3T12 cells were added. Normal resident peritoneal macrophages (N) and peptone-stimulated macrophages (S) were not tumoricidal in vitro (row A) and neither serum fraction-3 nor amphotericin B induced these macrophages to inhibit the growth of 3T12 cells as compared to growth in 3T12 control chambers (rows B–G). Macrophages obtained from animals with chronic BCG infection (A) were also nontumoral. These macrophage populations were activated, however, because unlike normal macrophages or stimulated macrophages, these macrophages had a marked cytotoxic effect on 3T12 target cells after a 2-hr preincubation with serum fraction-3 (row B). This 40-80 X 10^6-molecular weight fraction of mouse, fetal bovine, or human serum has been shown to render BCG macrophages tumoricidal in vitro (S). As can be seen, amphotericin B at 0.25–2 μg/ml also induced the BCG macrophages to become cytotoxic for 3T12 target cells in a dose-dependent manner. At the lowest concentration shown, 0.25 μg/ml (row C), there was only a slight cytotoxic effect (patchy areas of confluent target cell growth among the macrophages). At concentrations of 0.5–2 μg/ml (rows D–G) there appears to be complete inhibition of 3T12 cell growth among macrophage monolayers as compared to control 3T12 chambers. By light microscopy (×300) there was a marked cytotoxic effect (<5 3T12 cells per field).

In previous experiments the tumoral potential of nontumoral activated macrophages was found not to be sharply delineated but instead to be a modulable potential dependent on the net influence of antagonistic—both enhancing and inhibiting—stimuli. Over many experiments the tumoral potential of such activated but nontumoral macrophages harvested from animals with chronic BCG infection was observed to be somewhat variable. Tumor cell killing was found to occur only when the tumoral potential of the activated macrophage stimulated in vitro by BCG infection and in vitro by endotoxin reached a critically high level necessary for the expression of the cytotoxic capability of ac-

tivated macrophages (1). The extent of inhibition of 3T12 cell growth or cell lysis by BCG macrophages exposed to a particular concentration of amphotericin B was also found to be variable, especially with the lower concentrations of the polyene antibiotics. For example, amphotericin B at 0.5 μg/ml in 15 experiments induced a marked tumoricidal effect (<5 tumor cells per ×300 field); but in 5 experiments only a weak cytotoxic effect (5–30 cells per field) was seen and on 2 occasions only a cytostatic effect (30–40 cells per field) was observed. Thus, the responsiveness of nontumoral activated macrophages to amphotericin B in terms of acquisition of cytotoxic capability also seems highly dependent on the preexisting tumoral potential of the BCG macrophage population before exposure to the polyene antibiotics. Moreover, normal or stimulated macrophages never attain the critical tumoricidal potential necessary for tumor cell killing in response to even maximal nontoxic concentrations of amphotericin B (8 μg/ml).

The experiment illustrated in Fig. 2 compares the effect of amphotericin B with that of amphotericin B methyl ester on the tumoral capability of BCG-activated macrophages. Again, the adherent peritoneal cell monolayers (~90% macrophages) were incubated for 2 hr at 37°C with Dulbecco's modified Eagle's medium, amphotericin B, or amphotericin B methyl ester and then the polyeus were washed away before challenge with 3T12 cells. The BCG macrophages were nontumoral when cultured in 10% adult bovine serum (row A). Amphotericin B again induced the BCG macrophages to kill 3T12 cells in a dose-dependent manner ranging from a cytotoxic effect (row B) to a marked cytotoxic effect (rows C–D). At 4–8 μg of amphotericin B per ml, the BCG macrophages were lysed and the 3T12 cells grew to a thick multilayer (rows F–H). Amphotericin B methyl ester similarly induced a BCG macrophage cytotoxic effect for the 3T12 target cells. In comparison to amphotericin B, the methyl ester derivative induced a full cytotoxic effect over a broader concentration range (rows C–H) because this derivative was not toxic to BCG macrophages until concentrations >10 μg/ml.

The amphotericin B and its methyl ester (Fig. 2) used in the experiments shown were dissolved in dimethyl sulfoxide at a final concentration of 0.5% (vol/vol) in culture medium. The dimethyl sulfoxide itself had no effect on macrophage tumor cell killing or 3T12 cell growth. Amphotericin B suspended in deoxycholate (Fungizone®) was found to be equally potent.

FIG. 2. The experimental design is similar to that described for Fig. 1. Nontumoral BCG macrophages (A) were preincubated with either amphotericin B or amphotericin B methyl ester as indicated in the following concentrations (μg/ml): medium only, row A; 0.5, row B; 1.0, row C; 2.0, row D; 2.5, row E; 4.0, row F; 8.0, row G; 10.0, row H.
Although not shown, nystatin (Squibb) at 50-500 units/ml similarly enhanced BCG macrophage tumoricidal capability.

We have considered whether the polyene antibiotics exert their effect by rendering 3T12 target cells more susceptible to destruction by activated macrophages. Two lines of evidence in this is not the case. First, BCG macrophages adherent in microtiter chambers were induced to a tumoricidal state by a 2-hr exposure to amphotericin B or its methyl ester before 3T12 cells were present. Second, 3T12 cells grown overnight in medium supplemented with amphotericin B at 2 \( \mu \text{g/ml} \) and then washed twice with culture medium before addition to the cytotoxicity assay were no more susceptible to cell lysis by BCG macrophages induced to a tumoricidal state by endotoxin, by serum fraction-3, or by culture in endotoxin-free fetal bovine serum than were 3T12 cells grown in the absence of amphotericin B.

**Amphotericin B Induced Cloned Macrophages to Kill Tumor Cells.** We have examined the effect of amphotericin B on pure macrophage colonies to ascertain whether the observations described above actually are mediated by a direct effect of amphotericin B on macrophages. Macrophage colonies were cultured according to Stewart et al. (6). Such colonies when challenged with 3T12 target cells in 10% adult bovine serum are overgrown and dispersed by the 3T12 cells. Microscopically, the macrophages become rounded and displaced from the substrate. Incubation of these macrophages with lymphocyte derived MAF (1-25\%, vol/vol) prepared by periodate stimulation of mouse peritoneal exudate (5) cells did not induce any inhibition of 3T12 cell growth among the macrophage colonies and the colonies were similarly overgrown and dispersed (Fig. 3 A and B). Nontoxic concentrations of amphotericin B alone also were insufficient to induce cytotoxic activity among the macrophage colonies (not shown). However, when MAF (1\%, vol/vol) was combined with amphotericin B at 1 \( \mu \text{g/ml} \) and added to the cultures of 3T12 cells and cloned macrophages, the macrophage colonies maintained their integrity throughout a 60-hr assay and inhibited the growth of the 3T12 cells with which they were in contact (Fig. 3 C and D). These observations indicate that amphotericin B directly enhances the tumoricidal potential of activated macrophages.

Stewart et al. have recently shown that cloned macrophages can be induced to become cytotoxic for tumor cells and have confirmed by cinematography the actual lysis of tumor cells by cloned macrophages (7).

**Comparison of Amphotericin B with Other Polyene Antibiotics for Their Effects on Macrophage Tumoricidal Capability.** We have compared amphotericin B with two structurally different polyene antibiotics, filipin and pimaricin. Fig. 4 depicts a representative experiment comparing the tumoricidal inducing effects of these antibiotics on nontumoricidal activated macrophages. As shown in Fig. 4, BCG macrophages are not generally spontaneously tumoricidal when cultured in 10% endotoxin-free adult bovine serum. The addition of amphotericin B throughout the 60-hr assay induced the BCG macrophages to kill 3T12 target cells (rows C-F). Filipin and pimaricin, however, in comparable molar concentrations did not enhance the tumoricidal capability of BCG macrophages. In other experiments, filipin and pimaricin in maximal nontoxic concentration (5 \( \mu \text{g/ml} \) and 25 \( \mu \text{g/ml} \), respectively) were without effect on BCG macrophage tumoricidal potential. The polyenes in this experiment were dissolved in dimethyl sulfoxide which, as shown, did not account for the tumoricidal effect.

![Fig. 3](image-url) - Photographs of macrophage colonies in microtiter chambers after 6 hr of culture with 3T12 cells. The 3T12 cells are the larger cells with prominent nuclei. Macrophages cultured in 10% adult bovine serum with 1% MAF but without amphotericin B (A, \( \times 32; \) B, \( \times 96 \)) appear as darkly stained rounded cells displaced from their substrate by the 3T12 cell monolayer. In the presence of amphotericin B at 1 \( \mu \text{g/ml} \) and 1% MAF (C, \( \times 92; \) D, \( \times 123 \)) the macrophages in the central areas of the colony are rounded whereas in the peripheral areas they are fusiform and spread onto the substrate.

![Fig. 4](image-url) - In this experiment nontumoricidal BCG activated macrophages (A) were harvested from C57Bl/6 mice. Sixty-hour cultures were supplemented with 10% adult bovine serum and either amphotericin B, filipin, or pimaricin as indicated in the following molar concentrations: culture medium only, row A; 2.5 \( \times 10^{-10} \), row B; 5 \( \times 10^{-10} \), row C; 1 \( \times 10^{-9} \), row D; 2 \( \times 10^{-9} \), row E; 2.5 \( \times 10^{-9} \), and row F; 0.5% (vol/vol) dimethyl sulfoxide, row G. The concentration ranges on a weight basis (\( \mu \text{g/ml} \)) are amphotericin B (0.23-2.3), filipin (0.17-2.0), and pimaricin (0.17-2.0). Similar results were observed with pimaricin from two sources (Mycocard-Delft and Lederle Laboratories).
The distinguishing structural features of these polypeptide antibiotics are important to consider. Amphotericin B and nystatin are 37-carbon macrocyclic ring structures containing seven and six double bonds, respectively, while filipin and pimaricin are 27- and 25-carbon rings, respectively, containing five double bonds (8, 9). Filipin is neutral whereas the other polypeptides have charged amphipathic structures. Within membranes, amphotericin B and nystatin form relatively small aggregates with membrane cholesterol. Evidence to date suggests that such polypeptide-cholesterol complexes within the inner and outer halves of the lipid bilayer interact to form transmembrane complexes (9). These complexes organize in such a way as to create channels or pores selectively ionophoretic for small cations and anions (10, 11). Analysis of channel selectivity in *Acholeplasma laidlawii* membranes indicate passage of Na\(^+\), K\(^+\), Li\(^+\), and glucose but not Ca\(^{2+}\), Mg\(^{2+}\), or sucrose through the pores (11, 12). Filipin also binds to cholesterol within membranes with reportedly similar stoichiometry but does not induce selective membrane permeability changes. It has been proposed that the smaller dimension of the pimaricin molecule precludes the interaction of inner and outer membrane complexes and hence the formation of transmembrane channels (9). Similarly, filipin binds to membrane cholesterol but, unlike the charged polypeptides, forms large cholesterol aggregates visible by electron microscopy completely within the hydrophobic domains of the lipid bilayer. Filipin–cholesterol complexes are hence more disruptive to the membranes and, like pimaricin, do not induce selective permeability changes (9, 11).

**Influence of Other Ionophores on the Tumoricidal Capability of Activated Macrophages.** We have considered whether the enhancing effect of amphotericin B is dependent upon its ionophoretic action. Such an explanation would account for the ineffectiveness of filipin and pimaricin because these polypeptides are not ionophoretic. We have attempted to reproduce the amphotericin B effect with two polypeptide ionophores, gramicidin and alamethicin, whose action is dependent on membrane insertion and transmembrane channel formation but not upon binding of membrane cholesterol (13, 14). Gramicidin has been shown to inhibit platelet vesicle transport of 5-hydroxytryptamine, a process dependent upon a transmembrane electrochemical gradient (15). In artificial lipid bilayers gramicidin can dissipate such a gradient. Alamethicin likewise creates channels ionophoretic for cations and anions across artificial lipid bilayers and mitochondrial membranes (16). In nontoxic concentrations (Table 1) gramicidin at 1–5 \(\mu\)g/ml and alamethicin at 1–60 \(\mu\)g/ml had no effect on BCG-activated macrophage tumoricidal capability. We have also examined two ionophores that function by a carrier type mechanism: nigericin and valinomycin. Valinomycin has been shown to induce K\(^+\) efflux across mouse macrophage plasma membranes (17). Similarly, nigericin promotes K\(^+\) efflux and K\(^+\) for H\(^+\) exchange across biological membranes (18). Again in nontoxic concentrations valinomycin (0.05–0.1 \(\mu\)g/ml) and nigericin (0.1–0.5 \(\mu\)g/ml) were without effect on BCG-activated macrophage tumoricidal potential. These results along with the results obtained with the several polypeptide antibiotics tested are summarized in Table 1. We are not completely confident that any of the ionophores we have examined functionally reproduce the putative amphotericin B channels in macrophage plasma membranes and this explanation remains a possible one. Nevertheless, these experiments suggest that the ionophoretic properties of amphotericin B and nystatin do not account for their enhancing effect on macrophage tumoricidal potential.

**Table 1. Lack of correlation between the ionophoretic properties of polypeptide antibiotics and other ionophores and their ability to enhance activated macrophage tumor-cell killing**

<table>
<thead>
<tr>
<th>Agent*</th>
<th>Concentration, (\mu)g/ml</th>
<th>Ionophoretic action</th>
<th>Tumor cell killing†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin</td>
<td>0.5–1.5</td>
<td>Channel former</td>
<td>+</td>
</tr>
<tr>
<td>Nystatin</td>
<td>50–500</td>
<td>Channel former</td>
<td>+</td>
</tr>
<tr>
<td>Filipin</td>
<td>1–5</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Pimaricin</td>
<td>1–25</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Gramicidin</td>
<td>1–6</td>
<td>Channel former</td>
<td>0</td>
</tr>
<tr>
<td>Alamethicin</td>
<td>1–60</td>
<td>Channel former</td>
<td>0</td>
</tr>
<tr>
<td>Valinomycin</td>
<td>0.01–0.1</td>
<td>Carrier</td>
<td>0</td>
</tr>
<tr>
<td>Nigericin</td>
<td>0.1–0.5</td>
<td>Carrier</td>
<td>0</td>
</tr>
</tbody>
</table>

* All agents were preincubated with nontumoricidal BCG-activated macrophage monolayers for 2 hr at 37\(^\circ\) and washed away prior to the 60-hr cytotoxicity assay.
† +, denotes the induction of a marked cytolytic effect (<5 3T12 cells/×300 field) by BCG macrophages on 3T12 cells.
‡ Nystatin (Squibb) in injectable form is packaged as biological units of activity per milliliter, not \(\mu\)g/ml.

**DISCUSSION**

Activated macrophages obtained from animals with chronic infection or macrophages activated *in vitro* by lymphocyte- or monocyte- derived MAF possess a high potential for *in vitro* destruction of tumorigenic cells (19–21). Activated macrophages are not necessarily tumoricidal, however. Recent experiments indicate that, at least from the point of view of populations of macrophages, macrophage differentiation toward tumoricidal effector cells is not an all-or-none phenomenon but rather is differentiated within a continuum of an increasing potential for tumor cell lysis. Within this continuum we have recognized several functional states: normal, stimulated, nontumoricidal activated, and tumoricidal activated macrophages (1). The observations reported here further define such a conceptual framework. Amphotericin B was found to induce the full expression of tumoricidal capability in previously nontumoricidal but activated macrophages, yet this agent had no such effect on either stimulated or normal macrophages. Similarly, amphotericin B did not make cloned macrophages kill tumor cells unless MAF was also present. Qualitative changes in macrophage physiology incurred *in vivo* during an immunologic response or *in vitro* after exposure to MAF appear to be a prerequisite to the expression of the amphotericin B effect. We suggest that such alterations in macrophage function concomitant with activation are dependent upon modifications in the lipid content and organization of macrophage plasma membranes. The observation that activated (either nontumoricidal or tumoricidal) macrophages were much more susceptible to lysis by amphotericin B than were normal macrophages (>1.5 \(\mu\)g/ml vs. >8 \(\mu\)g/ml) supports such a hypothesis. Other investigators have demonstrated that differential susceptibility to eukaryotic cell lysis by amphotericin B is dependent on membrane cholesterol/phospholipid molar ratios and phospholipid fatty acid compositions (22, 23). Our results thus indicate that macrophage differentiation to the stage of nontumoricidal activation is associated with functional and structural membrane differentiation that renders the cell responsive to separate environmental signals for further differentiation to the tumoricidal state. In the absence of this membrane differentiation, such factors as amphotericin B or serum fraction-3 do not induce in the macrophage the critically high tumoricidal potential necessary for the destruction of tumorigenic cells.

We have considered the mechanism by which amphotericin
B renders BCG macrophages tumoricidal. As shown in Table 1, a number of other ionophores did not reproduce the amphotericin B effect. We have also considered whether binding of membrane cholesterol per se could enhance macrophage tumoricidal potential. However, the reported stoichiometries for cholesterol binding within membranes by the several polyene antibiotics tested here are all similar (11) and indicate that removal of membrane cholesterol from its binding to other membrane components is insufficient to enhance the tumoricidal potential of activated macrophages. This conclusion is in agreement with our unpublished observations that incubation of nontumoricidal activated macrophages with either lecithin liposomes or lipoprotein-depleted serum (density > 1.21 g/ml), both of which have been shown to remove cholesterol to a limited extent from erythrocyte and fibroblast membranes (24, 25), does not enhance the tumoricidal potential of nontumoricidal activated macrophages.

An alternative possibility is that transmembrane cholesterol-polyene complexes induce alterations in membrane organization with consequent changes in catalytic or receptor membrane functions. Of the polyenes studied, only amphotericin B and nystatin would be expected to form such transmembrane complexes. The insertion of hydrophobic protein into membrane or clustering of other lipids within membranes is known to influence the packing and composition of surrounding membrane lipids and in some cases induce phase separations or alterations in membrane protein distribution (26, 27). Such modifications in membrane organization could be expected to modify membrane function as well. Although the precise structural changes after complex formation are unclear, these observations define an agent that directly induces macrophage tumor cell killing in vitro for which the complete molecular structure is known. If the Amphotericin B effect has a physiologic correlate, we suggest that penetration of macrophage membranes by hydrophobic terminal complement proteins might, instead of lysis, induce tumoricidal activity. We have no direct evidence that this is the case, however.

Amphotericin B is the major therapeutic agent used in systemic mycotic infections. Our results indicate that concentrations of amphotericin B in the range of those achieved with standard therapy and to which most fungi are susceptible (28) also enhance activated macrophage tumoricidal capability. In view of the known parallels between acquisition of enhanced microbicidal and tumoricidal capability by macrophages it is possible that part of the therapeutic effect of amphotericin B operates through potentiation of macrophage fungicidal capability.

Recent evidence indicates that amphotericin B either alone (29) or in combination with the cytocidal drug 1,3-bis(2-chloroethyl)-1-nitrosourea may retard the progression of certain experimental and human tumors (30, 31). Many tumors contain substantial proportions of macrophages (32, 33), though the functional capabilities of such macrophages have not been widely studied. In at least one situation, progressing Moloney sarcomas, macrophages isolated from the tumors were found to be “primed” or nontumoricidal activated macrophages (34). If one assumes that macrophages within tumors other than Moloney sarcomas are also nontumoricidal but are activated, then the observed resistance to progression of neoplasia in vivo by administration of amphotericin B may operate in part via modification of macrophage tumoricidal capabilities. Our observation that the less toxic amphotericin B methyl ester has a similar enhancing effect (Fig. 2) on macrophage tumoricidal potential offers the possibility that this or other less toxic analogs of amphotericin B could be useful adjuvants in cancer therapy.

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