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Activation of pulmonary macrophages for fungicidal activity by gamma-interferon or lymphokines

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SUMMARY

The ability of murine recombinant γ interferon (IFN) or lymphokines to enhance the fungicidal activity of murine pulmonary macrophages (PuM) was studied in *in vitro*. PuM monolayers were incubated overnight with IFN, lymph node cells (LNC) plus concanavalin A, supernatants from Con A stimulated LNC or spleen cell cultures (Con A Sup), or tissue culture medium (TCM) $\pm$ Con A (5 μg/ml) or $\pm$ lipopolysaccharide (LPS, 10 ng to 10 μg/ml). After treatment, culture fluids were removed and PuM were challenged for 4 h with the yeast-form *Blastomyces dermatitidis* or 2 h with *Candida albicans*. Inoculum colony forming units (CFU) of *B. dermatitidis* were significantly reduced by PuM treated with 1000 U/ml of IFN (25 $\pm$ 3%), Con A Sup (25 $\pm$ 3%) or LNC plus Con A (37–44%), but not by TCM, ConA or LPS. *Candida albicans* was killed by PuM treated with Con A Sup (33 $\pm$ 8%) or LNC plus Con A (30–43%), but not by TCM, Con A, or LPS, and the activity of Con A Sup was neutralized by anti-IFN antibody. *Candida albicans* was not significantly killed by PuM treated with IFN doses ranging from 1 to 10^5 U/ml; nor did addition of LPS to IFN, or prolonged (3 day) treatment with IFN, result in significant killing of *C. albicans* by PuM. However, IFN (100 U/ml) could activate resident peritoneal macrophages for significant candidacidal activity (63%). These data indicate that PuM can be activated for fungicidal activity, and that PuM differ from resident peritoneal macrophages with regard to induction of candidacidal activity by recombinant γ-IFN.

Keywords alveolar macrophages microbicidal phagocytes interferon lymphokines lipopolysaccharide

INTRODUCTION

Pulmonary macrophages (PuM) are the first line of defence against inhaled microbes. Under ordinary conditions PuM keep the alveoli, bronchi, and lungs free of micro-organisms. The bactericidal activity of PuM from humans (Cohen & Cline, 1971), rats (Welch, 1981), or mice (Jakab, 1976) is well documented; however, some bacteria do resist their attack, e.g. *Legionella pneumophilia* (Nash, Libby & Horowitz, 1984), *Nocardia asteroides* (Davis-Scibienski & Beaman, 1984), and *Mycobacterium tuberculosis* (Leake, Myrvik & Wright, 1984). In contrast, PuM fungicidal activity is absent or limited for such pulmonary pathogens as *Histoplasma capsulatum* (Kinberlin *et al.*, 1981), *Blastomyces dermatitidis* (Sugar, Brummer & Stevens, 1986), *Coccidioides immitis* (Beaman & Holmberg, 1980), *Cryptococcus neoformans* (Fromtling & Shadomy, 1982),

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Rhizopus oryzae spores (Waldorf, Levitz & Diamond, 1984), and *Aspergillus fumigatus* mycelia (Schaffner *et al.*, 1983; Waldorf *et al.*, 1984).

Activation *in vivo* of PuM for enhanced microbicidal activity against resistant microbes such as *N. asteroides* (Davis-Scibienski & Beaman, 1984), *Listeria monocytogenes* (Johnson *et al.*, 1975), *M. tuberculosis* (Leake *et al.*, 1984), *Candida albicans* (Lehrer *et al.*, 1980), and *H. capsulatum* (Kinberlin *et al.*, 1981) has been reported. In some studies lymphokines were reported to activate PuM for enhanced bactericidal (Johnson *et al.*, 1975; Gomez *et al.*, 1985) or bacteriostatic (Nash *et al.*, 1984) activity. Although the activation of PuM for tumouricidal activity *in vitro* and *in vivo* with liposome-encapsulated muramyl dipeptide (Sone & Fidler, 1982) or lymphokines (Sone & Fidler, 1980) has been demonstrated, a direct role for interferon (IFN) in this process has not been demonstrated (Bordignon *et al.*, 1980; Akagawa & Tokunaga, 1985). Whereas a recent study (Murray *et al.*, 1985) indicated that human PuM can be activated for microbicidal activity by IFN, responsiveness of PuM from different species to the same stimulus is known to differ (Akagawa & Tokunaga, 1985).

Our previous studies of PuM from mice immunized subcutaneously with *B. dermatitidis* indicated that they did not have enhanced ability to kill it (Sugar *et al.*, 1986). Nevertheless, such immunized mice were highly resistant to pulmonary challenge with yeast-form *B. dermatitidis* (Morozumi, Brummer & Stevens, 1982), and PuM may be activated at the site of immunological reactions and thus play a role in host defence. The purpose of the present study was to test this hypothesis by studying the ability of various agents to enhance the fungicidal activity of PuM *in vitro*.

MATERIALS AND METHODS

Animals. Male BALB/cByJIMR mice, 8 to 12 weeks old, were obtained from the breeding colony of the Institute for Medical Research, San Jose, CA.

Reagents and media. Tissue culture medium RPMI-1640 with L-glutamine, heat-inactivated fetal bovine serum (FBS), and penicillin-streptomycin (P/S, 10,000 U/ml and 10,000 μg/ml, respectively) were obtained from GIBCO Laboratories (Grand Island, NY). Complete tissue culture medium (CTCM) consisted of 89 ml of RPMI-1640, 10 ml of FBS and 1 ml of P/S. Concanavalin A (Con A) was purchased from Sigma Chemical Co. (St Louis, MO) and lipopolysaccaride-W (LPS), prepared from *Escherichia coli* 0128:B12, was obtained from Difco Laboratories (Detroit, MI). Half-area 96-well tissue culture plates were supplied by Costar Co. (Cambridge, MA). Murine gamma-IFN, produced by recombinant DNA technology, and hyperimmune rabbit antibody (0·15 mg/ml) made against murine γ-IFN was provided by Genentech Corp. (South San Francisco, CA). The method of preparation, activity, and specificity of this antiserum have been described previously (Sverdersky *et al.*, 1984). Neutralizing activity of the antisera was measured by the ability to neutralize antiviral units (Sverdsky *et al.*, 1984; Brummer, Morrison & Stevens, 1985).

Fungi. Blastomyces dermatitidis ATCC 26199, an isolate which is virulent in mice, was used in all experiments, except one where other isolates previously studied, ATCC 26198, ATCC 26197 and an attenuated mutant of ATCC 26199 (Brass *et al.*, 1982a, b) were tested. Inocula of *B. dermatitidis* were prepared from cultures grown for 72 h on blood agar plates at 37°C. *Candida albicans* (isolate Sh27; ATCC 56882) (Scherer & Stevens, 1987) and a clinical *Candida parapsilosis* isolate were grown in yeast nitrogen broth (Difco) at 35°C for 3 to 4 days. The fungi were washed twice in 4 ml of saline, pelleted cells were suspended in CTCM, and units (single or multicellular) were counted with a haemacytometer. Viable colony forming units (CFU) were determined by plating 1 ml of an appropriate dilution in quadruplicate on blood agar plates.

Pulmonary macrophages. Lungs of mice were lavaged with Dulbecco's phosphate-buffered saline without calcium or magnesium (PBS) containing 0·1% ethylenediaminetetraacetic acid (EDTA) as previously described (Sugar, Brummer & Stevens, 1983). Cells obtained by repeated 1 ml lavages (total 12 ml/mouse) were pelleted by centrifugation (400 ***g***, 10 min). Pelleted cells were pooled, washed once in CTCM, and counted with a haemacytometer. A yield of $8{\cdot}5 \pm 2{\cdot}1 \times 10^5$ cells/

mouse, highly enriched for macrophages (97·5 ± 3·2%), were obtained by this method. PuM monolayers were formed by incubating 0·1 ml of lavaged cells ($2·5 \times 10^6$/ml CTCM) per microtest plate well for 2 h at 37°C in 5% CO_2–95% air humidified atmosphere. After this incubation non-adherent cells were aspirated and monolayers rinsed once with CTCM. Ninety percent of the incubated cells ($0·25 \times 10^6$) adhered and monolayers contained approximately $0·22 \times 10^6$ macrophages per well.

Supernatants. Spleen cells and lymph node cells (LNC) were harvested from normal mice and cultured as previously described (Morozumi *et al.*, 1982). Briefly, 5×10^6 LNC or spleen cells/ml CTCM were incubated (37°C, 5% CO_2–95% air), with or without Con A (5 μg/ml), for 24 h in 24-well tissue culture plates (2 ml/well). Cell-free supernatants were obtained by centrifugation of pooled cultures (400 ***g***, 10 min) followed by passage through 0·45 micrometer filters (Millipore Corp., Bedford, MA). Portions of supernatants were stored at −70°C.

Lymph node cells. Lymph nodes (axillary, brachial, inguinal and popliteal) were harvested from normal mice and single cell suspensions prepared as previously described (Morozumi *et al.*, 1982). LNC suspended in CTCM were used in co-cultures with PuM.

Treatment of macrophages and challenge. Macrophage monolayers were incubated overnight (37°C, 5% CO_2–95% air) with 0·1 ml of CTCM or CTCM containing 1 to 100,000 U of IFN/ml, Con A (5 μg/ml), LPS (10 ng to 10 μg/ml), undiluted Con A spleen cell or LNC culture supernatants, or LNC ($2·5 \times 10^4$ to 10×10^4/culture) plus Con A (5 μg/ml). After incubation, culture supernatants were aspirated and monolayers challenged for 2 h with 0·1 ml of *C. albicans* or *C. parapsilosis* (10,000 CFU/ml), or 4 h with *B. dermatitidis* (5,000 CFU/ml) plus 0·01 ml of fresh mouse serum at 37°C in 5%CO_2–95% air. Whereas *C. albicans* is phagocytized under these conditions (Sugar *et al.*, 1983a), *B. dermatitidis* yeasts of this isolate have been demonstrated to be too large to be phagocytized (Sugar *et al.*, 1986). Cultures were harvested by aspiration and repeated washing of culture wells with distilled water. Microscopic examination of culture wells indicated that there was complete removal of macrophages. Each culture and well washings were contained in a final volume of 10 ml. The number of CFU/culture was determined by plating 1 ml of the 10 ml distilled water dilution on a blood agar plate (BAP). Colonies of *Candida* were counted after 2 days and *B. dermatitidis* after 4 days of incubation at 35°C.

Statistics. Comparisons between groups were analysed by the Student's *t*-test, with significance assumed to be $P < 0·05$.

RESULTS

Effect of co-culturing PuM with LNC plus ConA. When LNC were co-cultured overnight with PuM in the presence of ConA (5 μg/ml), the fungicidal activity of PuM against *B. dermatitidis* and *C. albicans* were enhanced (Table 1). PuM incubated with CTCM, or CTCM plus LNC alone, were not able to significantly reduce inoculum CFU of *B. dermatitidis*; this was also true of PuM + CTCM + Con A (data not shown). In contrast, PuM incubated with 10^5 LNC plus Con A exhibited significant ability to kill *B. dermatitidis* (37%) ($P < 0·001$; Table 1). Incubation of fewer LNC ($0·25 \times 10^5$ or $0·50 \times 10^5$) plus Con A with PuM did not produce this effect (data not shown). PuM candidacidal activity was significantly enhanced ($P < 0·01$) when PuM were incubated with LNC plus Con A (43%). Similar results were obtained in a second experiment in which PuM plus LNC plus Con A resulted in PuM killing of *B. dermatitidis* (44%; $P < 0·002$) or *C. albicans* (30%; $P < 0·002$).

These results suggest that the stimulation of at least 10^5 LNC with Con A produced an effect on co-cultured PuM resulting in significantly enhanced fungicidal activity.

Effect of supernatants on fungicidal activity of PuM. Supernatants from 24 h spleen cell cultures incubated, with or without ConA (5μg/ml), were tested for their effect on PuM fungicidal activity. The results are given in Table 2. PuM incubated with Con A spleen cell supernatants had significantly enhanced ability to kill *B. dermatitidis* (28%) and *C. albicans* (24%). PuM incubated with CTCM, or control supernatants with Con A (5 μg/ml) added, did not have this effect (Table 2). In each of several experiments, PuM treated with Con A supernatants (two different batches)

Table 1. Activation of PuM for fungicidal activity by co-culturing with lymph node cells plus Con A

	CFU ± s.d.*	Percent reduction inoculum CFU ± s.d.	$P <$ †
Blastomyces dermatitidis +			
CTCM (0 h)	457 ± 60	—	—
CTCM (4 h)	480 ± 98	< 0	NS
PuM‡	437 ± 57	4	NS
PuM + LNC	440 ± 57	4	NS
PuM + LNC + ConA	290 ± 40	37	0·001
Candida albicans +			
CTCM (0 h)	473 ± 55	—	—
CTCM (2 h)	480 ± 49	< 0	NS
PuM	475 ± 7	< 0	NS
PuM + LNC	493 ± 38	< 0	NS
PuM + LNC + ConA	273 ± 66	43	0·01

*Mean CFU ± standard deviation of quadruplicate cultures.

†Comparisons with inoculum (fungus in CTCM, 0 h).

‡Pulmonary macrophage monolayer cultured overnight.

CTCM, complete tissue culture medium plus 10% fresh mouse serum. NS, not significant ($P > 0·05$).

LNC, lymph node cells, 10^5 per culture.

Con A, 5 μg Con A/ml.

Table 2. Activation of PuM for fungicidal activity with supernatants from Con A stimulated spleen cells

	CFU ± s.d.*	Percent reduction inoculum CFU	$P <$ †
Blastomyces dermatitidis +			
CTCM (0 h)	505 ± 70	—	—
CTCM (4 h)	495 ± 85	2	NS
PuM‡	567 ± 74	0	NS
PuM + supernatants§ + ConA‖	542 ± 67	0	NS
PuM + Con A supernatant¶	366 ± 15	28	0·01
Candida albicans +			
CTCM (0 h)	480 ± 50	—	—
CTCM (2 h)	510 ± 33	0	NS
PuM	493 ± 71	0	NS
PuM + supernatants§ + Con A‖	500 ± 87	0	NS
PuM + Con A supernatant¶	367 ± 72	24	0·05

*Mean CFU ± standard deviation of quadruplicate cultures.

†Comparisons with inoculum CFU (fungus in CTCM, 0 h).

‡Pulmonary macrophage monolayer cultured overnight.

§Supernatant from spleen cells cultured for 1 day.

‖ConA added to control supernatant to a final concentration of 5 μg/ml.

¶Supernatants from spleen cell cultured for 1 day in the presence of 5 μg Con A/ml.

CTCM, complete tissue culture medium plus 10% fresh mouse serum.

NS, not significant ($P > 0·05$).

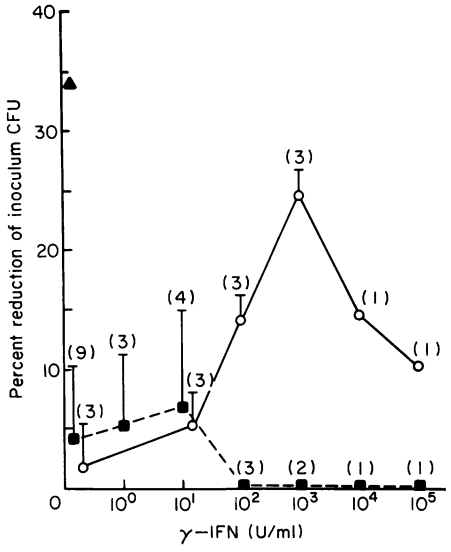

Fig. 1. Effect of γ-interferon on fungicidal activity of PuM. Mean percent reduction of inoculum colony forming units ± standard deviation of *Candida parapsilosis* (▲), *Blastomyces dermatitidis* (○) and *C. albicans* (■) by PuM after overnight incubation with varying concentrations of γ-IFN is given. The number of experiments per point is shown in parentheses.

significantly ($P < 0{\cdot}05$) killed *B. dermatitidis* (mean ± s.d., 25·3 ± 3%; $n = 3$) or *C. albicans* (33 ± 8%; $n = 5$). Moreover, treatment with supernatants from 24 h Con A stimulated LNC cultures also enabled PuM to significantly ($P < 0{\cdot}05$) kill *B. dermatitidis* (32%) or *C. albicans* (22%). These data indicate that Con A stimulated spleen cells or LNC produce soluble factors capable of inducing in PuM an enhanced ability to kill *B. dermatitidis* and *C. albicans*.

Effect of IFN on PuM fungicidal activity. PuM were incubated with CTCM or 1 to 10^5 U of IFN/ml CTCM, then challenged with *B. dermatitidis* or *C. albicans*. The results from such experiments are given in Fig. 1. PuM treated with only CTCM were able to kill *C. parapsilosis* (34%), but not significant numbers of *C. albicans* (4·4 ± 6·0%, $n = 9$) or *B. dermatitidis*. However, PuM treated with IFN (1000 U/ml) significantly reduced inoculum CFU of *B. dermatitidis* (25 ± 3%). In contrast, PuM treated with IFN did not exhibit significantly enhanced ability to kill *C. albicans* (Fig. 1). LPS alone (0·01–10 μg/ml, studied in 10-fold dilutions) could not consistently induce fungicidal activity against *B. dermatitidis* in PuM (range of 0–2% killing, $n = 1$ or mean of 2 at each dilution).

Effect of other IFN treatments and of LPS on candidacidal activity of PuM. Since human PuM have been reported to be activated by incubation with human IFN (300 U/ml) for 3 days (Murray *et al.*, 1985), similar experiments were done with murine PuM. In two experiments, treatment of PuM with IFN (100, 200, or 300 U/ml) for 3 days (even when media in one experiment were changed daily) did not enable PuM to significantly reduce inoculum CFU of *C. albicans*. In other experiments, when PuM were treated overnight with 10 or 20 IFN U/ml plus 5 ng LPS/ml, concentrations shown to enhance PuM cooperatively for tumorcidal activity (Akagawa & Tokunaga, 1985), they were not able to significantly kill *C. albicans*. No concentration of LPS (0·01–100 μg/ml, studied in 10-fold dilutions) used alone consistently activated PuM for candidacidal activity (range of 0–3% killing, $n = 1$ or mean of 2 at each dilution). Finally, LPS (5 ng/ml) added to Con A spleen cell supernatants did not increase the capacity of Con A supernatants to activate PuM for candidacidal activity, e.g. Con A supernatant 35%; Con A supernatant plus LPS, 32%.

Different effect of IFN on PuM and peritoneal macrophages. We have previously reported that resident peritoneal macrophages can be activated with IFN (1 to 10 U/ml) for significant killing of *C. albicans* (44 ± 12%) (Brummer *et al.*, 1985). To compare the effect of IFN on PuM to that of IFN on peritoneal macrophages, macrophage populations were isolated from the same mice and treated

Table 3. Comparison of IFN treatment of PuM vs peritoneal macrophages candidacidal activity

	CFU ± S.D.*	Percent reduction inoculum CFU	$P <$ †
Candida albicans +			
CTCM (0 h)	480 ± 50	—	—
CTCM (2 h)	510 ± 33	< 0	NS
PuM‡	493 ± 71	< 0	NS
PuM + IFN (100 U/ml)§	467 ± 73	3	NS
PeM‖	506 ± 66	< 0	NS
PeM + IFN (100 U/ml)	177 ± 27	63	0·001

*Mean CFU ± standard deviation of quadruplicate cultures.

†Comparisons with inoculum CFU (fungus in CTCM, 0 h).

‡Pulmonary macrophages cultured overnight.

§Recombinant murine γ-interferon.

‖Resident peritoneal macrophages cultured overnight.

CTCM, complete tissue culture medium plus 10% fresh mouse serum.

NS, Not significant ($P > 0·05$).

simultaneously with IFN. Treated macrophage monolayers were challenged with *C. albicans* for 2 h and the reduction of inoculum CFU per culture determined. The results from this experiment are given in Table 3. IFN treated peritoneal macrophages, but not PuM, had potent candidacidal activity (63%).

Killing of different B. dermatitidis *isolates by IFN activated PuM.* Isolate ATCC 26199 is virulent in mice compared to ATCC 26197, ATCC 26198, and attenuated ATCC 26199 (Brass *et al.*, 1982a, b). We tested the possibility that attenuated and less virulent isolates of *B. dermatitidis* would be more susceptible to killing by activated PuM than virulent ATCC 26199. The results from two experiments showed different isolates were killed (mean ± s.d. as follows: ATCC 26199, 32·5 ± 10·0%; ATCC 26199 attenuated, 35·0 ± 5·0%; ATCC 26198, 33·0 ± 10·0%; and ATCC 26197, 34·0 ± 2·5%. Although IFN activated PuM killed some isolates more efficiently than others, no correlation was seen between the virulence of an isolate and the extent of killing by IFN activated PuM.

Effect of anti-IFN antibody on Con A supernatant activation of PuM. We showed that Con A supernatants, but not recombinant IFN, could activate PuM for significant candidacidal activity. To address the question of whether the activating factor(s) in Con A supernatants were IFN, PuM were treated with Con A supernatants plus enough anti-IFN antibody to neutralize 200 U of IFN. Although Con A spleen cell supernatant-treated PuM killed *C. albicans* (27%; $P < 0·02$), the presence of anti-IFN antibody completely negated this effect. Similarly, the ability of ConA LNC supernatants to enhance the candidacidal activity of PuM (22% killing; $P < 0·01$) was abrogated after incubation of supernatants with anti-IFN antibody. Since anti-IFN antibody was not toxic for PuM, i.e. did not affect their ability to kill *C. parapsilosis*, these results suggest that PuM candidacidal activating factor(s) in Con A supernatants crossreacted with anti-recombinant γ-IFN antibody and thus were neutralized. Furthermore, the capacity of Con A LNC or spleen cell supernatants to activate PuM for killing *B. dermatitidis* (killing of 32% and 26%, respectively, $P < 0·05$) was negated by treating the PuM with supernatant and anti-IFN antibody sufficient to neutralize 1,125 U IFN/ml. Since 1,000 U IFN/ml activated PuM to kill *B. dermatitis* (mean 25 ± 3% killing, $P < 0·05$ in each of three experiments), these results suggest the major activating factor in Con A Sup was γ-IFN.

Effect of pH 2 on activity of Con A supernatants. Activate Con A supernatants were dialysed against 20 volumes of a glycine-HCl buffer pH 2, or PBS pH 7·4, for 24 h. This was followed by two successive 24 h dialyses against 20 volumes of CTCM. Treated supernatants were tested for their

effect on the candidacidal activity of PuM. We found that exposure of Con A supernatant to pH 2 buffer, but not to pH 7·4 buffer, abrogated its ability to activate PuM for candidacidal activity.

DISCUSSION

We report here that cultured PuM failed to kill yeast-form *B. dermatitidis in vitro*; however, PuM could be induced to reduce significantly the inoculum CFU by exposure to 1,000 U/ml of IFN, Con A supernatants, or LNC plus Con A. In this respect, PuM are similar to resident peritoneal macrophages which can also be activated by IFN or Con A supernatants (Brummer *et al.*, 1985), as well as co-culture with LNC plus Con A (unpublished observations) to kill *B. dermatitidis*. Moreover, the major activating factor for peritoneal macrophages in Con A supernatants was neutralized by anti-murine γ-IFN antibody, implying that the factor was γ-IFN (Brummer *et al.*, 1985). Although activation of PuM for tumoricidal activity (Sone & Fidler, 1980), bacteriostatic action (Nash *et al.*, 1984), or bactericidal action (Gomez *et al.*, 1985) by lymphokines has been reported, activation of murine PuM by IFN, to our knowledge, has not been reported. Moreover, it has been reported that murine PuM are not activated for tumoricidal activity by γ-IFN (Akagawa & Tokunaga, 1985).

The concentration of IFN (1,000 U/ml) required to activate PuM or resident peritoneal macrophages to kill nonphagocytizable *B. dermatitidis* was much greater than that needed to activate murine peritoneal macrophages (10 U/ml) to kill *C. albicans* (Brummer *et al.*, 1985), tumour cells (Sverdersky *et al.*, 1984) or *Trypanosoma cruzi* (125 U/ml for 3 days) (Wirth *et al.*, 1985). It remains to be determined whether exposure of PuM to lower concentrations of IFN for longer periods of time, e.g. 3 days, would activate PuM for fungicidal activity against *B. dermatitidis*. This may approach a more physiological situation, where sustained stimulation of PuM at an immunological reaction site could activate PuM for fungicidal activity.

IFN activated PuM were able to kill four different isolates of *B. dermatitidis*. However, no correlation was seen between the virulence of the isolate in mice and the extent of killing by IFN activated PuM. The basis for virulence of ATCC 26199 apparently does not lie in its resistance to killing by IFN-activated PuM or peritoneal macrophages (Brummer *et al.*, 1985) or a cell-free hydrogen peroxide-peroxidase-halide system (Sugar *et al.*, 1983b).

PuM had limited candidacidal activity unless they were activated by Con A supernatants or co-cultured with LNC plus Con A. Even treatment of murine PuM for 3 days with IFN did not activate PuM to significantly kill *C. albicans*. This contrasts with activation of human PuM to inhibit replication of *Toxoplasma gondii* or *Chlamydia psittaci* by a similar treatment with human IFN (Murray *et al.*, 1985). However, differences between species and targets may account for contrasting results. Furthermore, addition of LPS to IFN did not result in activation of PuM for significant candidacidal activity after overnight treatment. Unlike resident peritoneal macrophages, PuM were not activated for significant candidacidal activity by IFN, even by prolonged treatment. This difference between PuM and peritoneal macrophages in response to IFN is another example of how macrophages isolated from different anatomical sites may vary, e.g. phagocytosis (Hearst, Warr & Jakob, 1980), microbicidal activity (Lehrer *et al.*, 1980; Schaffner *et al.*, 1983), complement receptors (Blusse-VanOudAlblas & VanFurth, 1979; Hearst *et al.*, 1980; Weinberg & Unanue, 1981), enzyme content (Cohn & Wiener, 1963; Hearst *et al.*, 1980), and response to other stimuli (Leu *et al.*, 1972; Ryning, Krahenbuhl & Remington, 1981; Akagawa & Tokunaga, 1985). A recent preliminary report (Kemmerich, Rossing & Pennington, 1986) indicated that human γ-IFN does not activate human PuM for candidacidal activity, consistent with our findings.

We found that the activating factor(s) in Con A supernatants that enhanced PuM candidacidal activity was neutralized by anti-IFN antibody and inactivated at pH 2 (which inactivates γ-IFN). Since recombinant IFN did not have this enhancing effect, these findings suggest that: (a) natural γ-IFN, slightly different from recombinant IFN, was present in Con A supernatants; (b) for PuM candidacidal activity, γ-IFN is required but is not sufficient; or (c) PuM responded to a non-IFN factor in Con A supernatants that crossreacts with anti-IFN antibody. The last possibility has been suggested in the induction of tumoricidal activity in a macrophage cell line, where the synergistic

action of T cell-derived supernatants, with undetectable γ-IFN activity, was inhibited by antiserum raised against recombinant γ-IFN (Haman & Krammer, 1985). With respect to the first possibility, differences in glycosylation, and other differences, have been reported between natural and recombinant interferon molecules (Vilcek *et al.*, 1985).

There are reports that treatment of human PuM with LPS overnight induces enhanced tumoricidal activity (Sone *et al.*, 1982; Sverdersky *et al.*, 1985). We found that exposure of murine PuM to LPS (or, as stated above, IFN plus LPS) failed to induce enhanced fungicidal activity against *C. albicans*, nor could LPS substitute for IFN for enhancement of fungicidal activity against *B. dermatitidis*. These differing results with LPS probably reflect the vast differences in target cells (tumours vs. fungi), and possibly the mechanism by which the target cells are killed. Other workers (Agawa & Tokunaga, 1985) have reported that LPS fails to activate murine PuM for tumoricidal activity.

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