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Activation of Murine Polymorphonuclear Neutrophils for Fungicidal Activity with Supernatants from Antigen-Stimulated Immune Spleen Cell Cultures

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An in vitro model of in vivo immunological activation of murine polymorphonuclear neutrophils (PMN) was developed. Culture supernatants of spleen cells from *Blastomyces dermatitidis*-immunized mice stimulated with *B. dermatitidis* antigens in vitro were studied. Incubation of the supernatants with thioglycolate-elicited PMN enabled the cells to significantly reduce (31 ± 6%) *B. dermatitidis* inoculum CFU. Optimum production of active supernatants occurred after 4 to 6 days of stimulation in vitro and required 200 μg of nonviable *B. dermatitidis* cells per ml. Generation of activity by immune spleen cells was shown to be antigen specific in that stimulation with a heterologous antigen or stimulation of nonimmune spleen cells with *B. dermatitidis* antigen did not produce active supernatants. The activity in supernatants was dose dependent, nondialyzable (molecular weight ⩾ 14,000), and relatively heat labile (80°C, 30 min). Activation of PMN by supernatants for fungicidal activity against *B. dermatitidis* required only a short incubation period (1 h) followed by a 2-h coculture (challenge) period. Stimulation of normal spleen cells with concanavalin A also resulted in the production of supernatants capable of activating PMN for significant fungicidal activity (31.1 ± 8.5%). These findings demonstrate for the first time a link between soluble factors produced by antigen stimulation of sensitized lymphoid cells and activation of PMN for enhanced microbicidal activity. Such a process defines an additional immune defense mechanism whereby the immune host may clear specific microorganisms.

It has been reported that 29 to 47% of the peripheral blood polymorphonuclear neutrophils (PMN) from patients with gram-negative bacterial infections reduced Nitro Blue Tetrazolium compared with 8.5% of PMN from healthy donors(19, 24, 25). These observations called attention to the fact that under certain conditions, e.g., infection (19) or phagocytosis in vitro (3), the metabolic activity of PMN could be modulated. More recently it has been shown that chemotactic factors, such as C5a and *N*-formyl methionyl-leucine-phenylalanine, enhanced the metabolic activity of PMN as measured by increased hexose monophosphate shunt activity (13, 18), chemiluminescence (5, 28), and superoxide anion production (28). This activation correlated with increased killing of nonopsonized *Escherichia coli*, *Streptococcus faecalis*, or *Staphylococcus aureus* (18). Furthermore, supernatants from phytohemagglutinin-stimulated human peripheral blood mononuclear cells reportedly enhanced PMN hexose monophosphate shunt activity, reduction of Nitro Blue Tetrazolium (20), and killing of nonopsonized *Serratia marcescens* (11). These latter observations imply an important link between stimulated lymphocytes and positive modulation of PMN metabolic activity.

Previously, we have reported that PMN elicited with homologous antigen intraperitoneally (i.p.) in *Blastomyces dermatitidis*-immune mice acquired enhanced candidacidal activity and ability to kill *B. dermatitidis* in vitro (8; E. Brummer, A. M. Sugar, and D. A. Stevens, J. Leukocyte Biol., in press). The in vivo activation of PMN was immunologically specific and implied that the interaction of antigen with sensitized lymphocytes resulted in an influx of PMN and enhancement of their microbicidal activity and suggested that lymphokines may be involved in this process. The purpose of the present study was to develop an in vitro model of the in vivo immunological activation of PMN and with this model characterize and study spleen cell culture supernatants with ability to "activate" normal PMN for fungicidal activity against *B. dermatitidis*.

MATERIALS AND METHODS

Animals. Pathogen-free BALB/cByJIMR male mice (Institute for Medical Research, San Jose, Calif.) 8 to 12 weeks of age were used in these experiments.

Fungi. *B. dermatitidis* ATCC 26199, a strain shown to be virulent in mice (16), was used throughout these studies. Log-phase yeast-form *B. dermatitidis* from liquid medium (16) 72-h cultures was used to inoculate blood agar plates (BAP). Inocula for in vitro challenge were prepared by harvest of 48 h of growth from BAP, which was washed twice in saline and resuspended, and multicellular units were counted with a hemacytometer. Appropriate dilutions of suspended inocula were plated in quadruplicate on BAP to determine the number of CFU per milliliter. *B. dermatitidis* (yeast form) stored at −70°C for more than 2 years were nonviable and served as killed *B. dermatitidis* cells.

Candida albicans Sh27 was grown in yeast nitrogen base broth (Difco Laboratories, Detroit, Mich.) at 32°C from stock cultures stored on Sabouraud agar slants at 4°C. *C. albicans* cells grown in yeast nitrogen base broth for 4 days at 32°C were washed twice in saline and counted with a hemacytometer. The CFU per milliliter of inoculum was determined by plating 1 ml of an appropriate dilution in quadruplicate on BAP.

Media and reagents. Minimal essential medium, RPMI 1640, heat-inactivated fetal bovine serum, penicillin (10,000 U/ml), streptomycin (10,000 μg/ml) and Dulbecco phosphate-buffered saline were purchased from GIBCO Laboratories, Grand Island, N.Y. Complete tissue culture medium

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(CTCM) consisted of RPMI 1640, 10% (vol/vol) fetal bovine serum, and 100 U of penicillin plus 100 μg of streptomycin per ml. Metrizamide [2-(3-acetamido-5-*N*-methylacetamido-2,4,6 triiodobenzamido)-2-deoxy-D-glucose] (Sigma Chemical Co., St. Louis, Mo.) was made up as a 35.5% isotonic solution in distilled water. The required concentrations of metrizamide for gradients were made by diluting the 35.5% solution in phosphate-buffered saline.

Immunization of mice. Mice were given 20,000 CFU of *B. dermatitidis* subcutaneously at each of two dorsal sites. As reported previously (22), resolution of this infection over a period of 4 weeks rendered mice resistant to fatal pulmonary challenge. Resistance correlated with lymphocyte proliferative responses, specific serum antibodies, and cutaneous delayed hypersensitivity reactions to *B. dermatitidis* antigens (22). Such mice are referred to as immune mice and were used in experiments 4 weeks postinfection.

PMN. Peritoneal exudate cells (PEC) enriched for PMN were induced by injecting normal mice with 1 ml of thioglycolate broth (Clinical Standards Laboratories, Carson, Calif.) i.p. 4 h before harvest. In selected experiments, PEC were induced in immune mice with 500 μg of killed *B. dermatitidis* cells in 0.5 ml of saline i.p. 24 h before harvest. PEC were collected by repeated lavage of the peritoneum with a total of 10 ml of minimal essential medium containing 10 U of preservative-free heparin (American Scientific Products, McGaw Park, Ill.) per ml. PEC were washed once with minimal essential medium, suspended in CTCM, and counted with a hemacytometer. PMN were purified as previously described (6). Briefly, thioglycolate-induced PEC (85% PMN, 11% lymphocytes, 4% monocytes-macrophages) were layered on a metrizamide gradient (1.5 ml of 14.5% metrizamide over 1.5 ml of 15.5% metrizamide) and centrifuged at 400 × *g* for 20 min. Layer-1 cells at the interface (24% PMN, 68% lymphocytes, 8% monocytes-macrophages) were aspirated, and pelleted cells (90% PMN, 8% lymphocytes, 2% monocytes-macrophages) (pellet-1 cells) were washed once with minimal essential medium and suspended in CTCM (6).

Supernatants. Spleen cells were harvested from normal or immune mice and cultured as previously described (22). Briefly, 10×10^6 or 20×10^6 spleen cells per ml of CTCM were stimulated with 0, 100, 200, or 500 μg of killed *B. dermatitidis* cells (whole killed cells [WKC]) per ml in 24-well tissue culture plates, 2 ml per well (Falcon no. 3047; Falcon Plastics, Oxnard, Calif.). Cultures were harvested after 2, 4, or 6 days at 37°C in a humidified 5% CO_2–95% air atmosphere. In some cultures of *B. dermatitidis*-sensitized spleen cells, 200 μg of heat-killed *C. albicans* per ml was substituted for *B. dermatitidis* antigen. For supernatants from mitogen-stimulated spleen cell cultures, normal spleen cells, 10×10^6 per ml of CTCM, were stimulated with concanavalin A (ConA) (0, 1, 5, or 10 μg/ml of CTCM), 2 ml per well in 24-well tissue culture plates, for 1, 2, or 3 days. Cell-free supernatants were obtained by centrifugation of pooled cultures (400 × *g*, 10 min) followed by filtration through 0.45-μm filters (Millipore Corp., Bedford, Mass.). Portions of supernatants were stored at −70°C until needed.

Cocultures of PMN and *B. dermatitidis* or *C. albicans*. In experiments in which PEC were induced in immune mice by i.p. injection of killed *B. dermatitidis* cells, PMN-enriched populations (pellet-1 cells) were dispensed (0.1 ml of 5×10^6/ml of CTCM) in flat-bottom wells of 96-well MicroTest plates (Linbro, Flow Laboratories, Hamden, Conn.) and then challenged with 0.1 ml of *B. dermatitidis* or *C. albicans* (5,000 or 10,000 CFU/ml of CTCM) plus 0.02 ml of fresh mouse serum. Cocultures were incubated in a humidified atmosphere of 5% CO_2–95% air at 37°C for 2 h. Cultures were harvested with distilled water as described previously (6), and the number of CFU per culture was determined by plating on BAP. The percent reduction of inoculum CFU was calculated by the formula: [1-(coculture CFU/inoculum CFU)] × 100. Student's *t* test was used for comparisons between groups. Microscopic examination of harvested cocultures indicated that reduction of CFU in cocultures was not due to clumping, e.g., the mean number of cells per multicellular unit was similar in control and experimental cultures.

Activation of PMN in vitro. Thioglycolate-induced PEC (85% PMN) or metrizamide gradient pellet-1 cells (90% PMN) from normal mice were suspended in supernatants (5×10^6/ml), dispensed (0.1 ml per flat-bottom well), incubated for 1 h at 37°C in 5% CO_2–95% air, and then challenged with 0.1 ml of *B. dermatitidis* (5,000 CFU/ml) or *C. albicans* (10,000 CFU/ml) plus 0.02 ml of fresh normal mouse serum. After 2 h at 37°C in 5% CO_2–95% air, cocultures were harvested, and CFU per culture were determined as described above.

Treatment of supernatants. Supernatants from sensitized spleen cell cultures stimulated with 200 μg of WKC of *B. dermatitidis* for 6 days were treated with heat in a water bath at 60 or 80°C for 30 min. Supernatants were concentrated threefold with a Minicon S-125 apparatus (Amicon Corp., Danvers, Mass.). Undiluted or concentrated supernatants were dialyzed at 4°C against 10 volumes of CTCM for 48 h (5 volumes for 24 h and another fresh 5 volumes for 24 h). For these dialysis studies, Spectra/Por 2 tubing with a molecular weight cutoff of 12,000 to 14,000 (Spectrum Medical Indus-

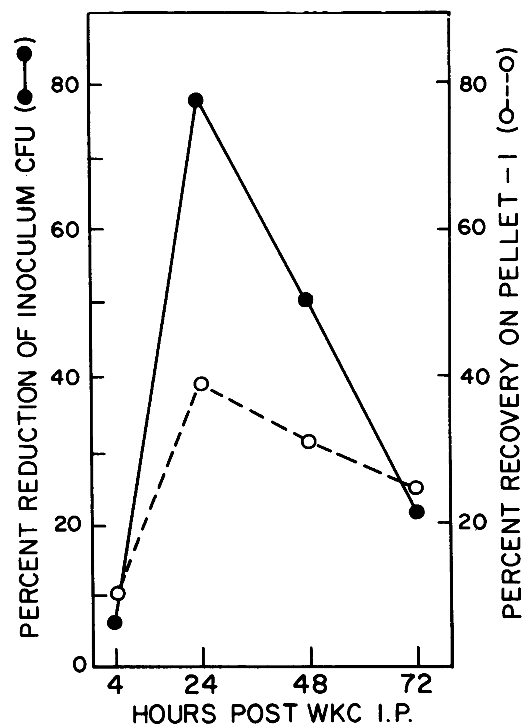

FIG. 1. Effect of time after i.p. injection of WKC on activation of PMN in immune mice. We show the percent reduction of inoculum CFU (●) and percent recovery of PEC in the pellet of metrizamide gradients (○).

tries, Inc., Los Angeles, Calif.) was gently boiled in sterile distilled water for 30 min before use.

RESULTS

Effect of time after i.p. injection of killed cells on activation of PMN in immune mice. A group of immune mice was given 500 μg of killed *B. dermatitidis* cells i.p., and PEC were harvested from groups of three mice 4, 24, 48, or 72 h later. The yield of PEC per mouse increased with time after i.p. injection from 4.4×10^6 (4 h) to 20×10^6 (72 h) per mouse; however, the yield of pellet-1 cells from metrizamide gradients was highest (39%) when PEC were harvested 24 h after treatment (Fig. 1). Furthermore, when pellet-1 cells were tested for fungicidal activity against *B. dermatitidis*, those isolated from PEC present 24 h after treatment had the highest activity, i.e., 78% reduction of inoculum CFU (Fig. 1). Fungicidal activity in pellet 1 cells was still high (50%) at 48 h and significant (21%) at 72 h, but not significant after only 4 h of antigen exposure in vivo (Fig. 1). These results indicate that the process of activation requires more than 4 h, reaches a peak about 24 h after exposure to antigen, and then declines. Although the greatest number of PEC were present at 72 h, the PMN-enriched population from these PEC did not have the greatest fungicidal activity. These findings suggest the PMN as isolated in pellet-1 are optimally activated 24 h after treatment of immune mice i.p. with WKC.

Effect of time postimmunization on activation of PMN in vivo. Groups of three mice immunized 4, 6, or 8 weeks earlier were given 500 μg of killed *B. dermatitidis* cells i.p., and PEC were harvested 24 h later. When metrizamide gradient pellet-1 cells were tested for activity against *B. dermatitidis*, the greatest activity (90%) was found in cells isolated 4 weeks postimmunization (Fig. 2). Significant activation was still achievable 6 and 8 weeks postimmunization, e.g., 60% reduction of inoculum CFU (Fig. 2). The number of PEC induced with 500 μg of WKC was greatest (20×10^6 per mouse) 4 weeks postimmunization and then declined to 12×10^6 per mouse 6 and 8 weeks postimmunization (Fig. 2).

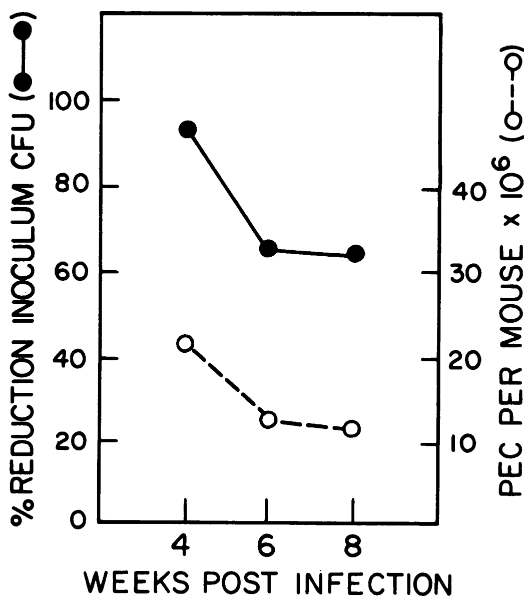

FIG. 2. Effect of time postimmunization on activation of PMN by i.p. injection of WKC 24 h earlier. Shown are percent reduction of inoculum CFU (●) and the mean yield of PEC per mouse (○).

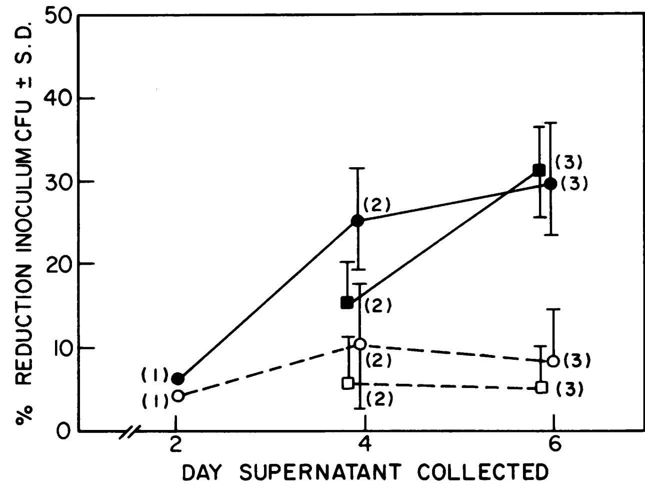

FIG. 3. Activation of normal PMN with supernatants. Effect of supernatants from cultures of 10×10^6 (●) or 20×10^6 (■) antigen-stimulated (200 μg of WKC per ml) immune spleen cells and 10×10^6 (○) or 20×10^6 (□) unstimulated immune spleen cells on percent reduction of inoculum CFU by PMN. The mean percent reduction of CFU ± standard deviations (vertical bars) of separate experiments (number shown in parentheses) testing the same batch of supernatants are given.

These findings are consistent with previous work (22) which showed that peak immunological responses (antigen-induced blastogenesis and delayed hypersensitivity reactions) were optimal 4 weeks postimmunization and then declined. They also suggest that PMN could be activated 24 h after immunized mice were challenged with this pathogen and consequently play a major role in the resistance of mice to reinfection.

Activation of normal PMN with supernatants from antigen-stimulated immune spleen cells. PMN elicited i.p. with thioglycolate in normal mice had highly significant fungicidal activity against *C. albicans* but not against *B. dermatitidis* (8). Consequently, such cells were ideal for testing various supernatants from spleen cell cultures for ability to activate them (render them fungicidal for *B. dermatitidis*). When supernatants from cultured immune spleen cells (10×10^6 or 20×10^6/ml) stimulated with 200 or 500 μg, but not 100 μg, of WKC per ml for 2, 4, or 6 days were tested, ability to activate was found only in supernatants from 4- and 6-day cultures (Fig. 3). Supernatants from the same spleen cells not stimulated with antigen had some ability to activate PMN, but this was not significant ($P > 0.05$) compared with CTCM (Fig. 3). Supernatants from antigen-stimulated immune spleen cells collected after 6 days of culture significantly ($P < 0.01$) increased the fungicidal activity of PMN (30 ± 5%) compared with control supernatants. Three different supernatant preparations tested in different experiments had similar activity. When metrizamide gradient-purified PMN (>90% PMN) were treated with active supernatants, results similar to those above were obtained. None of the supernatants incubated alone with *B. dermatitidis* reduced inoculum CFU (data not shown). These results indicate that only supernatants from antigen-stimulated immune spleen cells contained soluble factors which acted on normal PMN to render them significantly fungicidal for *B. dermatitidis*.

Antigen specificity in production of active supernatants. Supernatants from 6-day cultures of immune spleen cells stimulated with a heterologous antigen (heat-killed *C. albi-*

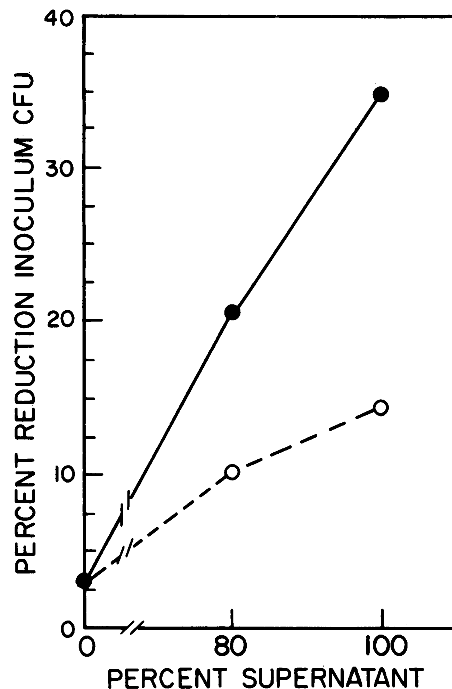

FIG. 4. Effect of supernatant concentration on activation of normal PMN. The percent reduction of inoculum CFU by normal PMN treated with supernatants from antigen-stimulated (●) and unstimulated (○) immune spleen cell cultures is shown.

cans, 200 μg/ml) failed to activate PMN for fungicidal activity against *B. dermatitidis*. Furthermore, supernatants from 6-day cultures of normal spleen cells stimulated with 200 μg of WKC per ml did not have the ability to activate PMN (data not shown). Taken together, these and the above data indicate that only immune spleen cells stimulated with specific antigen produced supernatants after 4 to 6 days of culture, which, when incubated with normal PMN, enabled the PMN to significantly reduce inoculum CFU of *B. dermatitidis*.

Effect of supernatant concentration on activation of PMN. When dilutions of supernatants from 6-day immune spleen cell cultures stimulated with 200 μg of WKC per ml were used to treat normal PMN, it was found that undiluted supernatants were the most effective in activating PMN for fungicidal activity against *B. dermatitidis* (Fig. 4). For example, PMN incubated in undiluted supernatant for 1 h and then challenged for 2 h were able to reduce inoculum CFU by 35%, whereas PMN in 80% supernatant or in CTCM had less activity, 21 and 3%, respectively. Furthermore, when active supernatants were concentrated threefold the activity was increased more than twofold (Table 1). These results demonstrate a dose-dependent effect in active supernatants.

Preliminary characterization of active supernatants. Supernatants from sensitized spleen cell cultures stimulated with 200 μg of WKC per ml for 6 days were treated in various ways to determine some characteristics of the active factors present. Activity in supernatants was partially destroyed by incubation at 60°C for 30 min and completely inactivated by 80°C for 30 min (Table 1). A second experiment with the same supernatant gave similar results. When active supernatants were dialyzed against CTCM, activity was not lost (Table 1). Furthermore, when active supernatants were concentrated two- to threefold and dialyzed against CTCM, the activity was increased over those of nonconcentrated supernatant two- to threefold (Table 1). In these same experiments, dialyzed and then concentrated supernatants activated pellet-1 cells (>90% PMN) for significant ($P < 0.05$) fungicidal activity against *B. dermatitidis* (data not shown). These results indicate that soluble factors capable of activating PMN are relatively heat labile, nondialyzable, and have molecular weights greater than 14,000.

Activation of normal PMN with supernatant from cultures of spleen cells stimulated with ConA. Since it has been

TABLE 1. Effect of concentration, dialysis, or heating on ability of supernatants to activate PMN[a]

Expt.	Treatment	CFU per well ± SD	% Reduction of inoculum CFU	Significance (P<)
1	Inoculum (0 h)	203 ± 5[b]	0	
	Inoculum +			
	PMN + CTCM[c]	207 ± 15	0	NS[d]
	PMN + control supernatant[e]	180 ± 44	11	NS
	PMN + experimental supernatant[f]	158 ± 5	22	0.001
	PMN + concentrated experimental supernatant[g]	105 ± 50	48	0.01
	PMN + dialyzed experimental supernatant[h]	158 ± 33	22	0.05
2	Inoculum (0 h)	560 ± 20[b]	0	
	Inoculum +			
	PMN + CTCM	560 ± 80	0	NS
	PMN + control supernatant	480 ± 60	14	0.05
	PMN + experimental supernatant	420 ± 60	25	0.01
	PMN + experimental supernatant, 60°C for 30 min	500 ± 100	11	NS
	PMN + experimental supernatant, 80°C for 30 min	560 ± 100	0	NS

[a] A 2-h PMN-fungus coculture.
[b] At 0 h, quadruplicate cultures.
[c] PMN treated with CTCM.
[d] NS, Not significant ($P > 0.05$).
[e] PMN treated with 6-day supernatants from unstimulated immune spleen cell cultures.
[f] PMN treated with 6-day supernatants from antigen-stimulated immune spleen cells.
[g] PMN treated with supernatant concentrated 2.7-fold and dialyzed against CTCM.
[h] PMN treated with supernatant dialyzed against 10-fold volumes of CTCM for 48 h.

reported that supernatants from mitogen-stimulated human lymphocyte cultures enabled human PMN to kill nonopsonized bacteria (20), we investigated the ability of supernatants from ConA-stimulated murine spleen cells to activate murine PMN for fungicidal activity against *B. dermatitidis*. PMN were incubated for 1 h with ConA-stimulated spleen cell culture supernatants which had been harvested after 1, 2, or 3 days of culture. This was followed by challenge with *B. dermatitidis* for 2 h. Only supernatants from 3-day cultures stimulated with 5 or 10 μg, but not 1 μg, of ConA per ml had the ability to induce significant fungicidal activity in PMN for *B. dermatitidis* (Fig. 5). Supernatants from nonstimulated spleen cell cultures or ConA alone did not exhibit this activity (Fig. 5). These data show that supernatants from spleen cell cultures stimulated with ConA contained soluble factors capable of activating PMN for significant ($P < 0.02$) fungicidal activity against *B. dermatitidis* (31.1 ± 8.5%) (Fig. 5).

DISCUSSION

Previous work (8) described activation of PMN in vivo for enhanced candidacidal activity (45% versus 90%) and ability to kill *B. dermatitidis* (0% versus 90%). Here we report that this process required more than 4 h, was optimal at 24 h, and correlated with the time after antigen exposure rather than with the number of cells elicited at those times. These kinetics after antigen exposure resemble those reported for the activation of resident peritoneal macrophages for tumoricidal (26), fungicidal (7), or microbicidal (14) activity after i.p. injection of ConA in normal mice or killed microorganisms in immune mice. This similarity in kinetics of activation suggests that common mechanisms and possibly lymphokines may be involved in both PMN and macrophage activation.

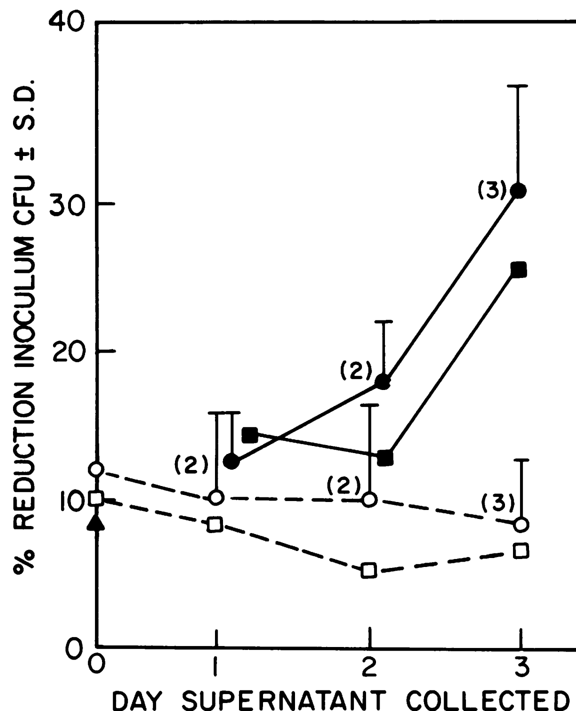

FIG. 5. Effect of supernatants from ConA-stimulated spleen cell cultures on activation of PMN. Shown are the mean percent reduction of inoculum CFU ± standard deviation (vertical bars) by PMN treated with supernatants from ConA-stimulated (5 μg/ml, ●; 10 μg/ml, ■) or unstimulated (○,□) spleen cells or CTCM plus 10 μg of ConA per ml (▲) in separate experiments (number shown in parentheses).

A plausible explanation for activation of PMN in vivo, subsequent to antigen-specific or mitogenic responses, was the action of elaborated lymphokines on resting PMN. When we tested this possibility with an in vitro model of in vivo immunological activation of PMN, supernatants from appropriately stimulated spleen cell cultures did indeed activate PMN. Relatively brief periods of exposure of PMN to supernatants, e.g., 1 h exposure plus 2 h in coculture, resulted in their activation. On the other hand, activation was not enhanced more by longer periods of exposure before coculture, e.g., 2 or 3 h (data not shown). This contrasts sharply with activation of peritoneal macrophages by supernatants, in which longer periods of exposure (12 h or more) were required for optimal results (2, 4, 10, 26). The significance of these differences in time required for activation by lymphokines is not clear, but they could reflect basic differences in the mechanisms involved or different durations of responsiveness and functional ability of these cell types in in vitro cultivation, or both.

The conditions necessary for the production of active supernatants by antigen stimulation of sensitized spleen cells correlated with those optimal for proliferative responses reported previously (22), e.g., 4 to 6 days in culture. In contrast, supernatants with macrophage activating factor have been generated by antigen stimulation of sensitized human peripheral blood mononuclear cells (2) or murine spleen cells (4, 10) over shorter culture periods, e.g., 2 or 3 days. However, a feature common to both systems was the antigen specificity in the production of active supernatants. Although preliminary results suggest that 6-day supernatants from antigen-stimulated sensitized spleen cell cultures as well as 3-day supernatants from ConA-stimulated spleen cells may also contain macrophage activating factor, additional experiments are required to clarify these observations. Our data indicate that supernatants harvested late in the incubation period contain the most activity for activating PMN, and these activating factors may be different from macrophage activating factor.

The activity in supernatants was shown to be dose dependent, and it could also be enriched by concentration. This characteristic will be valuable in future investigations aimed at purification, isolation, and biochemical characterization of active factors. Results from preliminary studies reported here show that the activty in supernatants was nondialyzable molecular weight ≥14,000) and heat labile (80°C, 30 min).

The activity in supernatants described here resembles that reported by Cross and Lowell (11) and others (15, 20) in supernatants from human peripheral blood mononuclear cells stimulated with phytohemagglutinin. Only relatively short periods (1 to 3 h) of PMN incubation with supernatants were required for enhancement of bactericidal activity, using a short challenge time (2 h). On the other hand, the relationship between factors in active supernatants reported here and the multitude of compounds reported to be capable of altering the metabolic activity of human (1, 9, 12, 21, 23, 27) or rat (17) PMN is not clear at this time.

The cellular source of active factors in supernatants responsible for activation of PMN has not been determined in the work presented here. However, we speculate that T lymphocytes are essential for the initiation of this process, but additional experiments are required to determine whether they or other lymphoid cells, e.g., B cells or monocyte-

macrophages, or both, produce active factors. Furthermore, we speculate that soluble active factors elevate PMN to an enhanced metabolic state in a manner described for several activating compounds (5, 9, 21, 27), namely, interaction with membrane receptors. As shown in this study and our previous report (8) on in vivo-activated PMN, enhanced killing by activated PMN appears linked with severalfold greater production of toxic products from oxidative metabolism after appropriate stimulation. The killing occurs with contact of effector cell and fungus, but not phagocytosis, because the CFU are too large for the PMN to ingest. The process of contact may also be enhanced by supernatant materials.

In summary, we report here for the first time that supernatants generated by stimulation of sensitized spleen cells with specific antigen contain factors capable of activating PMN for microbicidal activity. These findings in vitro and in vivo (8) demonstrate an important link between soluble factors produced by antigen stimulation of sensitized lymphoid cells and induction of enhanced microbicidal activity in PMN, resulting in an additional immune defense mechanism whereby the immune host could clear the specific microorganisms.

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