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Gamma Interferon Reverses Inhibition of Leukocyte Bactericidal Activity by a 25-Kilodalton Fraction from *Mycobacterium tuberculosis*

AHMED A. WADEE,[1]* JONATHAN D. COHEN,[2] AND ARTHUR R. RABSON[1]

Department of Immunology, The South African Institute for Medical Research,[1] Department of Medicine, Coronation Hospital,[2] School of Pathology, University of the Witwatersrand, and Human Cellular Immunology Unit, The South African Medical Research Council, Johannesburg 2000, South Africa

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In this study we examined the effects of *Mycobacterium tuberculosis* cell extracts on the phagocytic activity of polymorphonuclear leukocytes and cultured peripheral blood monocytes. *M. tuberculosis* cell extracts were fractionated on Sephacryl S-200 columns, and a 25-kilodalton glycolipoprotein was shown to inhibit the intracellular killing ability of these leukocytes but had no effect on their phagocytic potential. This same fraction inhibited fusion of phagosomes with lysosomes, as assessed by noting the transfer of acridine orange from lysosomes to phagosomes. This fraction was shown to have a maximal inhibitory effect when it was in the form of an intact carbohydrate-lipid-protein complex. Gamma interferon (IFN-γ), but not IFN-α, reversed the inhibitory effect of the mycobacterial component on bactericidal activity and on fusion of phagosomes and lysosomes. Thus, this 25-kilodalton fraction of *M. tuberculosis* cell extract may be important in protecting organisms against phagocytic degradation, an effect which can be reversed by IFN-γ.

An important mechanism leading to degradation of bacteria inside phagocytic cells involves the transfer of lysosomal enzymes to the phagocytic vacuole (1, 6). The studies of Axline and Cohn (3) and Cohn et al. (8, 9) have demonstrated the presence of large, dense, preexisting lysosomal granules inside macrophages which can fuse with and discharge their contents into the phagosomes (3, 8, 9). This results in the production of digestive vacuoles, or phagolysosomes, responsible for degradation of ingested materials (7).

Several species of intracellular pathogenic microorganisms have been shown to survive and escape this intracellular degradation. Included in this group are *Toxoplasma gondii* (24), *Mycobacterium microti* (21), *Chlamydia psittaci* (13), *Encephalitozoon cuniculi* (33, 38), *Mycobacterium tuberculosis* (2, 20), and *Legionella pneumophila* (22). With regard to mycobacteria, a number of factors may inhibit fusion of phagosomes with lysosomes (phagosome-lysosome fusion) (14, 16, 26, 27, 39); Gordon et al. (15) have demonstrated that *M. tuberculosis* culture filtrates contain ammonia, which is presumably liberated by the organisms and which substantially inhibits phagosome-lysosome fusion, probably by raising the pH within the phagosome. However, recent reports by Goren et al. (18, 19) indicate that some of these observations may be artifactual. This statement is based on their studies which indicate that polyanionic agents, one group of substances described as inhibitors of phagosome-lysosome fusion, may concentrate in lysosomes as gelatinous hydrocolloids that ionically trap acridine orange, the usual marker for measuring phagosome-lysosome fusion. This trapped acridine orange may not transfer to phagosomes for many hours.

In the present study we examined the role of sonicated mycobacteria on phagosome-lysosome fusion, as measured by the acridine orange method. During this study, reports indicating that the acridine orange technique could be artifactual were received. Therefore, the emphasis of our experiments changed, and we examined the effect of extracts of sonicated mycobacteria on the intracellular killing capacity of cultured monocytes and freshly prepared normal peripheral blood polymorphonuclear leukocytes (PMN) and in modulation by gamma interferon (IFN-γ). In this report, we demonstrate that mycobacterial sonic extracts can reduce the intracellular killing capacity of these phagocytic cells. This effect is principally due to the presence of a low-molecular-mass (25-kilodalton [kDa]) factor that could prevent the fusion of phagosomes with lysosomal granules.

MATERIALS AND METHODS

Establishment of monocyte monolayers. Peripheral blood monocytes were isolated from healthy volunteers and were maintained in culture by using a modification of the technique described by Johnson et al. (23). Mononuclear cells taken from Ficoll-Hypaque density gradients were washed in phosphate-buffered saline (PBS), and the cell pellet was resuspended in PBS containing 0.3 mM EDTA. Cells were washed two more times in this medium and suspended in RPMI 1640 (M. A. Bioproducts, Walkersville, Md.) containing 10% heat-inactivated autologous serum. Mononuclear cells at a concentration of 3×10^6/ml were plated in 16-mm wells of multidish Lab-Tek slides with plastic tissue culture chambers (Miles Scientific, Div. Miles Laboratories, Inc., Naperville, Ill.), each well containing 0.5 ml. Cell cultures were incubated for 30 min at 37°C in a humidified incubator containing 5% CO_2 in 95% air. At the end of this incubation period, the wells were washed three times with warm (37°C) Hanks balanced salt solution (HBSS) to remove nonadherent cells. The remaining adherent cells were then cultured in 1.5 ml of Modular Medium (Highveld Diagnostics, Johannesburg, South Africa) with 10% fresh autologous serum and 100 U each of penicillin and streptomycin. Culture medium

* Corresponding author.

was replaced every 4 days by removing half of the supernatant fluid and replenishing the remaining fluid with an equivalent amount of Modular Medium containing 10% serum. Growth of cells was examined with an inverted microscope, and cells were subcultured when the monolayer reached 75% confluency. Subculturing of cells was done by vigorous pipetting of cell cultures followed by plating into wells of equal size with an equal volume of medium containing 10% autologous serum. Cell viability was assessed by trypan blue exclusion, and cell cultures were usually maintained for 3 months or longer. Greater than 95% of these cells reacted with monoclonal antibody OKM1 (Ortho Diagnostics, Inc., Raritan, N.J.) and were actively phagocytic.

For bactericidal studies, cultured monocytes were removed from wells by treatment with trypsin-EDTA. The monolayer was washed with warm (37°C) PBS, 0.5 ml of a trypsin-EDTA mixture (0.125% trypsin, 0.05% EDTA, 0.1% glucose in calcium- and magnesium-free Dulbecco medium [National Institute of Virology, Johannesburg, South Africa]) was added to the monolayers, and the cultures were incubated at 37°C for 15 min. Cells were collected, pooled, and washed vigorously three times with cold RPMI containing 10% heat-inactivated fetal calf serum at 4°C. They were then counted and reconstituted to a concentration of 10^7/ml in PBS.

Isolation of PMN. PMN were obtained from the pellets produced in Ficoll-Hypaque density gradients. These pellets were suspended in 38 ml of PBS and mixed with 12 ml of 3% gelatin (Difco Laboratories, Detroit, Mich.) in PBS. The cell-gelatin suspension was incubated at 37°C for approximately 30 min, and the PMN-rich supernatant was removed and centrifuged, and the cell pellet was washed three times in PBS. Residual erythrocytes were lysed with 0.83% ammonium chloride at 4°C by the method of Boyle (5), and the remaining PMN were washed, counted, and suspended in PBS.

Mycobacterium preparation. *M. tuberculosis* cells were obtained by scraping colonies from Lowenstein-Jensen slopes. Organisms were washed, heat killed, homogenized, and counted, and the concentration was adjusted to 10^6/ml of PBS (pH 7.2). Mycobacterial extracts were obtained by sonication in an MSE 50-W ultrasonic disintegrator as described previously (37). The sonicated material was centrifuged at 2,000 × *g* for 10 min at 4°C, the supernatant was collected, and the amount of protein was determined by using the Bio-Rad protein estimation kit. This material was fractionated by applying 2.0 ml of concentrated protein onto precalibrated Sephacryl S-200 columns (Pharmacia, Uppsala, Sweden), with a flow rate of 1.0 ml/min. Fractions were collected in 2.0-ml aliquots, and protein elution profiles were plotted by measuring the amount of protein in each fraction.

Carbohydrate moieties were degraded by the method of Owhashi et al. (34). Lyophilized fractions were incubated in 0.05 M sodium metaperiodate in darkness with gentle mixing for 72 h at 4°C. The reaction was terminated by dialysis against four changes of PBS at 4°C. In some experiments, active fractions were treated with 1 μg of proteinase K per ml (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) for 1 h at room temperature to destroy proteins; this procedure was followed by extensive dialysis at 4°C.

Removal of lipid from these fractions was achieved by treating them with 2 volumes of anesthetic ether. The mixture was frozen at −70°C and thawed at 37°C, and the interface material, containing lipid, was removed. This process was repeated three times, after which lipid was no longer observed at the interface. Lipid samples were pooled, and the ether was removed by evaporation over nitrogen. The remaining lipid was reconstituted to the original volume in C-RPMI (RPMI containing 10% heat-inactivated fetal calf serum) containing 0.1 ml of ethanol and sonicated in an MSE 50-W ultrasonic disintegrator. The remaining non-lipid-containing fractions were reconstituted to the original starting volume, and both types of fractions were assessed for their ability to inhibit phagosome-lysosome fusion.

Phagocytosis of *Candida albicans* by monocytes and PMN. *C. albicans* was grown overnight in glucose-saline, washed three times in PBS, and killed by heating at 80°C for 1 h. They were then washed with PBS, resuspended in PBS to a concentration of 6×10^7/ml, and stored at −20°C until used. Concentrations of cultured monocytes or PMN were adjusted to 10^7/ml, and 0.3 ml of this suspension was incubated with 0.1 ml of the *C. albicans* suspension and 0.1 ml of fresh autologous serum. The nonphagocytic control consisted of 0.1 ml of *C. albicans* suspension, 0.1 ml of serum, and 0.3 ml of PBS. Cultures were incubated at 37°C on a revolving wheel for 50 min, and 0.2-ml aliquots were removed every 10 min and added to 0.5 ml of leukocyte counting fluid. Phagocytosis was expressed as the percentage of extracellular *C. albicans*, which were enumerated on a Neubauer hemacytometer, calculated by using the following formula: % phagocytosis = 100 − {[(no. of extracellular organisms)/(no. of organisms in the nonphagocytic control)] × 100}.

Phagosome-lysosome fusion. A small pellet of bakers' yeast (*Saccharomyces cerevisiae*) was suspended in physiological saline, and the concentration was adjusted to 10^6 viable yeast cells per ml. These cells were opsonized by incubating 10^6 cells in 5.0 ml of fresh serum for 30 min at 37°C, after which they were washed twice and added to acridine orange-labeled phagocytes in tissue culture chamber slides. Leukocytes were prelabeled by exposure to 5 μg of acridine orange per ml for 15 min at 37°C, followed by a further 15 min of incubation in HBSS at 37°C. They were then washed and reconstituted to 1.0 ml in HBSS. These prelabeled cells were incubated with the opsonized yeast cells at 37°C for 30 min and were then washed extensively with HBSS to remove nonphagocytosed yeast cells. Fusion was allowed to progress for a further 30 min at 37°C, and after a final wash the plastic chamber was removed from the slide and wet mounts were made and examined under a Leitz UV microscope. By using the criteria of Goren et al. (17), viability, nonviability, and intermediate killing of yeast cells were scored independently by two investigators. Yeasts that were regarded as having been killed appeared uniformly bright green. Cells in which fluorescence was restricted to the periphery of the cell were placed in the intermediate category, implying an early stage of phagosome-lysosome fusion. Yeasts that appeared nonfluorescent were regarded as not having acquired or retained acridine orange by lysosome-phagosome fusion. The latter observation implied that fusion had been delayed or inhibited.

In experiments in which the effects of mycobacterial sonic extracts or fractions on the fusion process were examined, these fractions were added to the monocyte cultures together with yeast cells at final concentrations of 50 and 100 μg/ml. In some experiments cultured monocytes or PMN were pretreated with IFN-γ (Amersham Corp., Bucks, England) or IFN-α (Roche Diagnostics, Div. Hoffman-La Roche Inc., Johannesburg, South Africa) for 1 h, after which time cultures were washed and reconstituted with RPMI 1640 containing 10% fetal calf serum and examined for phagosome-lysosome fusion.

TABLE 1. Effect of *M. tuberculosis* extracts on the phagocytosis of yeast particles

Time (min)	% Phagocytosis (range)[a] by:			
	PMN		Cultured monocytes	
	Without extract	With extract	Without extract	With extract
10	25 (22–28)	24 (21–26)	23 (21–25)	25 (22–27)
20	30 (28–32)	21 (19–23)	28 (26–30)	26 (24–30)
30	80 (75–89)	79 (77–80)	76 (22–78)	74 (70–76)
40	95 (90–98)	94 (92–96)	90 (88–94)	87 (86–88)
50	96 (95–97)	95 (93–98)	97 (93–99)	96 (94–98)

[a] Mean and range of three experiments.

Intracellular killing assays. Cultures of *Staphylococcus aureus* were grown overnight in brain heart infusion broth, washed three times in PBS, and resuspended in PBS to a final concentration of 10^8/ml. The bactericidal ability of PMN or monocytes was assessed by using a modification of the technique of Quie et al. (35). Each test system contained 0.5 ml (10^7/ml) of PMN or monocytes, 0.1 ml of *S. aureus*, 0.1 ml of fresh autologous serum with or without *M. tuberculosis* extracts, or 0.1 ml of interferon. The final volume was adjusted to 1.0 ml with PBS. Cell-free control systems contained 0.1 ml of serum, 0.1 ml of *S. aureus*, and 0.8 ml of PBS. Cultures were incubated at 37°C for 1.5 h on a rotating wheel. At the end of this incubation period, 0.1-ml aliquots were transferred to 9.9 ml of distilled water and then serially diluted in distilled water to 10^{-5}. A 0.1-ml portion of this dilution was plated onto nutrient agar, and the plates were incubated overnight at 37°C, after which time colonies were counted.

Colonies were then enumerated, and the difference in counts between test and control systems was assessed and expressed as the percentage of bacteria killed, calculated by using the following formula: % killing = 100 − {[(no. of colonies in test systems)/(no. of colonies in control system)] × 100}.

TABLE 2. Intracellular killing ability of PMN and cultured monocytes

Cell type/ expt no.	*S. aureus* mean colony count ± SD[a] (% killed) with:		
	Medium alone	Phagocytic cells	Phagocytic cells with *M. tuberculosis* extracts
PMN			
1	320	42 (87)	115 (64)
2	350	14 (96)	70 (80)
3	290	5 (98)	96 (67)
4	347	0 (100)	121 (65)
5	363	40 (89)	92 (75)
Mean ± SD[b]	334 ± 29	20 ± 20 (94)	99 ± 20 (70)
Cultured monocytes			
1	284	50 (80)	125 (56)
2	296	47 (87)	151 (49)
3	316	38 (88)	161 (49)
4	327	36 (89)	192 (41)
5	314	56 (82)	157 (49)
Mean ± SD[b]	307 ± 12	45 ± 8 (84)	157 ± 24 (49)

[a] Mean colony count ± standard deviation of five experiments.
[b] $P < 0.001$.

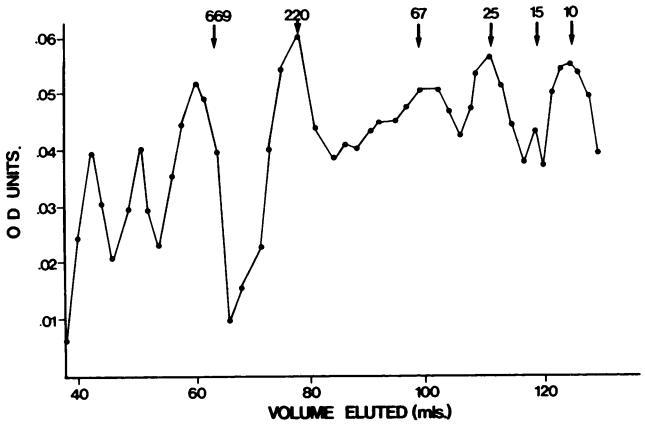

FIG. 1. Elution profile of *M. tuberculosis* extract which was passed through a Sephacryl S-200 column. Molecular mass markers (in kilodaltons) are indicated by arrows.

RESULTS

Effects of *M. tuberculosis* extracts on the phagocytosis of *C. albicans* by cultured monocytes and PMN. To determine whether soluble extracts of *M. tuberculosis* cells had any effect on the phagocytic capacity of leukocytes, leukocytes were incubated with *C. albicans* cells in the presence or absence of mycobacterial extracts for various periods of time. Mycobacterial extracts had no effect on the phagocytic capacity of either macrophages or PMN (Table 1).

Effects of mycobacterial extracts on intracellular killing capacity. Both cultured monocytes and PMN were capable of killing the majority of *S. aureus* cells 1.5 h after the initiation of the system. However, in the presence of soluble mycobacterial extracts a considerable decline in this intracellular killing ability was noted for both cell types (Table 2).

In order to characterize the component responsible for reducing the intracellular killing capacity of PMN, the soluble *M. tuberculosis* extract was passed through a Sephacryl S-200 column. The protein elution profile obtained is shown in Fig. 1. Major protein peaks obtained from these columns were pooled, concentrated, filter sterilized, and assessed for their ability to reduce the intracellular killing capacity of *S. aureus* cells. The results indicate that the reduction of intracellular killing of *S. aureus* was due to a 25-kilodalton (kDa) molecule (Table 3). Larger or smaller components of

TABLE 3. Effect of various fractions of *M. tuberculosis* extracts on the intracellular killing capacity of PMN[a]

Material incubated with PMN	Mean (range) % intracellular killing	% Change from medium control
Medium alone	94 (80–99)	
M. tuberculosis extract	50 (40–58)	44[b]
Fraction from Sephacryl S-200 columns (kilodaltons)		
640	93	1
220	90	4
50	92	2
25	46 (39–57)	48[b]
15	89	5
10	92	2

[a] Mean of three experiments.
[b] $P < 0.001$.

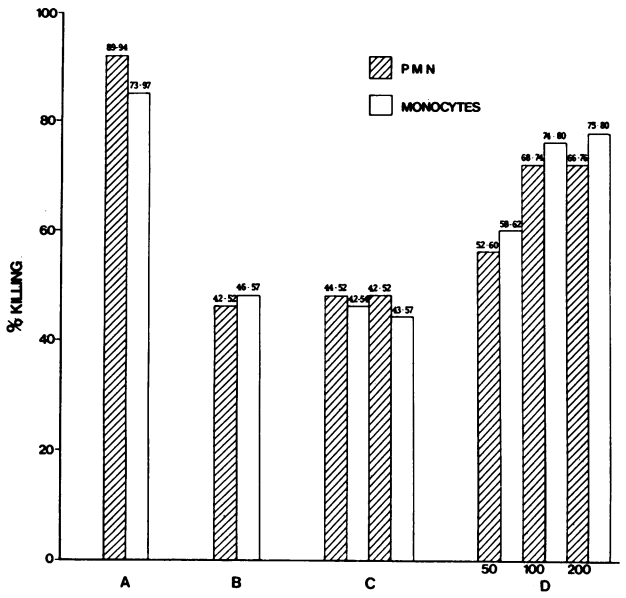

FIG. 2. Bactericidal activity of PMN and cultured monocytes in the presence of medium (A), the 25-kDa fraction (B), and of the 25-kDa fraction with IFN-α (C) and the 25-kDa fraction with increasing concentrations of IFN-γ (shown in units per milliliter at the bases of the bars) (D). The mean and range of three experiments are shown on the *y* axis and above the bars, respectively.

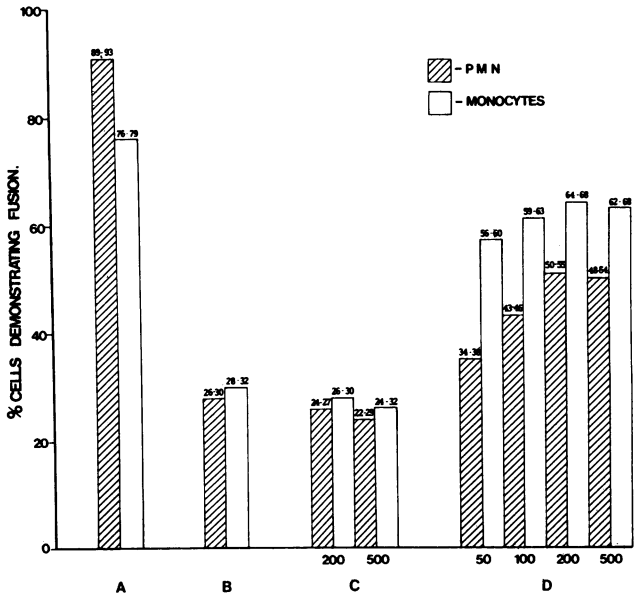

FIG. 3. Phagosome-lysosome fusion in PMN and cultured monocytes in the presence of medium (A), the 25-kDa fraction (B), and of the 25-kDa fraction with IFN-α (C) and the 25-kDa fraction with increasing concentrations of IFN-γ (shown in units per milliliter at the bases of the bars) (D). The mean and range of three experiments are shown on the *y* axis and above the bars, respectively.

M. tuberculosis extract fractions did not affect the intracellular killing capacity of PMN. When PMN were treated with the 25-kDa fraction in the presence of IFN-α or IFN-γ, significant recovery in bactericidal activity was shown by PMN treated with IFN-γ but not by those treated with IFN-α (Fig. 2). It should be noted that IFN-γ could not completely restore intracellular killing ability even when used at doses of 200 U/ml (data not shown).

Phagosome-lysosome fusion. Lysosomes of both PMN and cultured monocytes were prelabeled with acridine orange, and the phagocytes were permitted to ingest live bakers' yeast cells in the presence or absence of mycobacterial extracts or of the 25-kDa active component. A mean of five experiments confirmed that mycobacterial extracts and, in particular, the 25-kDa fractions from these extracts significantly inhibited phagosome-lysosome fusion (Table 4).

In some experiments, phagocytes were treated with either IFN-α or IFN-γ before those cells were tested for phagosome-lysosome fusion. The 25-kDa fraction significantly inhibited phagosome-lysosome fusion in phagocytes incubated with *C. albicans* (Fig. 3). However, when this fraction was added to the system in the presence of interferon, only IFN-γ significantly reversed the inhibitory effect. IFN-α, at a range of concentrations, had no effect. Neither form of interferon on its own influenced phagosome-lysosome fusion in phagocytes incubated with *C. albicans* (data not shown).

Chemical modification of the 25-kDa fraction from *M. tuberculosis* extracts. To further characterize the 25-kDa inhibitor of phagosome-lysosome fusion, molecules taken from Sephacryl columns were pooled, concentrated, and subjected to treatment that removed lipid or degraded carbohydrate or polypeptide portions. Removal of lipid, treatment with sodium metaperiodate, or, in particular, combinations of these two treatments reduced the majority of the inhibitory effect (Table 5). Degradation of protein with proteinase K only minimally reversed the inhibitory action

TABLE 4. Percentage of yeast-containing PMN and cultured monocytes demonstrating phagosome-lysosome fusion (as evidenced by dye uptake) in the presence or absence of *M. tuberculosis* extracts[a]

Yeast-containing-cell system	% Fused	% Not fused	% Intermediately fused
Monocytes	78	12	10
PMN	82	8	10
Monocytes + *M. tuberculosis* extract	37[b]	43	20
PMN + *M. tuberculosis* extract	32[b]	46	22
Monocytes + 25-kDa fraction	34[b]	49	17
PMN + 25-kDa fraction	30[b]	54	16

[a] Mean of five experiments.
[b] $P < 0.001$.

TABLE 5. Effect of chemically modifying the 25-kDa mycobacterial fraction on Phagosome-lysosome fusion of cultured monocytes[a]

Yeast-containing-cell system	% Fused	% Not fused	% Intermediately fused
Monocytes	79	11	10
Monocytes + untreated fraction	37	47	16
Monocytes + fraction treated with:			
Proteinase K	48	37	15
Sodium metaperiodate	58	30	12
Ether	60	28	12
Sodium metaperiodate + ether	70	25	5

[a] Mean of five experiments.

of the 25-kDa fraction. Therefore, these results indicate that the active components present in the 25-kDa fraction are probably glycolipoproteins.

DISCUSSION

The present study describes a 25-kDa mycobacterial glycolipoprotein that has the capacity to inhibit intracellular killing by monocytes and PMN but that has no effect on the phagocytic ability of these leukocytes. Macrophages infected with mycobacteria have previously been shown to ingest adequate numbers of killed, attenuated, or viable organisms (12, 29), and there is little or no evidence to suggest that mycobacteria possess antiphagocytic moieties on their surfaces (29). The results from the present study confirm that crude *M. tuberculosis* extracts do not affect the phagocytosis of yeast particles by leukocytes, or, alternatively, do not affect the adhesion of yeast particles to the phagocytes. Cree and Beck (10), however, demonstrated that PMN which had previously phagocytosed *Mycobacterium leprae* cells subsequently ingested fewer zymosan particles. This suggests that *M. tuberculosis* and *M. leprae* evade phagocyte bactericidal activity by different mechanisms (25, 40). *M. tuberculosis* extracts were capable of inhibiting the intracellular killing ability of both PMN and cultured monocytes. The ability of these leukocytes to kill *S. aureus* intracellularly was markedly depressed in the presence of mycobacterial sonic extracts. Although the possibility exists that these sonic extracts are toxic to the phagocytes, it is unlikely because they did not affect cell viability or phagocytic activity. In order to investigate the nature of the factor(s) responsible for this inhibition of killing activity, crude mycobacterial extracts were fractionated on Sephacryl S-200 columns. Of the protein peaks obtained, maximal reduction (46%) of intracellular killing of *S. aureus* was observed in the presence of the 25-kDa fraction. Similar inhibition of bactericidal activity was observed in cultured monocytes treated with this fraction (data not shown).

Of particular interest was the observation that IFN-γ, but not IFN-α, reversed the inhibitory effect of the 25-kDa fraction on the bactericidal activity of both PMN and cultured monocytes. IFN-γ has been identified as a lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity (32). Bhardwaj et al. (4) have shown that IFN-γ-activated monocytes inhibit the intracellular multiplication of *Legionella pneumophila*. Other workers have also demonstrated that IFN-γ increases the susceptibility of *T. gondii* (31, 32), *Leishmania donovani* (28, 30), and *Chlamydia psittaci* (36) to the antimicrobial effects of phagocytes. Although the mechanism by which IFN-γ reverses this effect is not known, Edwards et al. (11) have previously shown that the interaction between the host and mycobacteria is modulated profoundly by prostaglandins. They suggest that administration of macrophage-activating agents enhances resistance to mycobacterial infection by competing with prostaglandin E2.

Studies were also undertaken to assess phagosome-lysosome fusion. In these studies cultured monocytes or PMN were pretreated with acridine orange, which is concentrated in secondary lysosomes. The phagocytes were then allowed to ingest viable yeast cells, and phagosome-lysosome fusion was recognized by passage of the fluorescent marker into the yeast cell-containing phagosome. Fusion of lysosomal granules with phagosomes was viewed with UV light, under which killed organisms appeared a uniformly bright green color, whereas viable organisms failed to take up the dye. Our results indicate that mycobacterial extracts, and in particular the 25-kDa fraction from these extracts, inhibit phagosome-lysosome fusion and may be responsible for the reduction in intracellular killing ability.

Although the mechanism by which phagosome-lysosome fusion is inhibited is not known, it could involve dysfunction in phagosomal or lysosomal membranes or both. It has previously been shown that polyanions act on the secondary lysosomal membrane by actually binding to these membranes and presumably by interfering with critical transport processes (16).

With a view to assessing the effects of interferons on defective phagosome-lysosome fusion, IFN-γ and IFN-α were incubated with leukocytes which had been treated with the 25-kDa fraction. The results show that IFN-γ, but not IFN-α, reverses the inhibitory effect of the mycobacterial glycolipoprotein on phagosome-lysosome fusion. A major problem with these experiments, however, concerns recent reports which question the acridine orange technique for measuring phagosome-lysosome fusion owing to the fact that acridine orange is trapped by polyanionic substances (18, 19). Therefore, it is difficult to ascribe the defect in bactericidal killing to abnormal phagosome-lysosome fusion. However, the observation that IFN-γ reverses both the bactericidal effect and the transfer of acridine orange from the lysosomes to the phagosome suggests that these two functions are related.

Attempts to define the components that are inhibitory to phagosome-lysosome fusion revealed that degradation of protein molecules resulted in only minimal reversal of the inhibitory effect. When carbohydrate moieties were degraded, however, significant reversal of fusion was observed. Similarly, treatment of the 25-kDa fraction with ether resulted in a significant improvement in fusion. Therefore, preliminary chemical modification of this fraction indicates that the important constituents are carbohydrate and lipid moieties which are apparently in a complex with protein. It is not yet clear whether two separate molecules have been identified or whether these components of the 25-kDa fraction need to be in a complex for biological activity. From these results, it is apparent that maximal inhibitory activity is obtained from an intact 25-kilodalton molecule. Therefore, it is tempting to speculate that mycobacterial infection is associated with the release of this factor and others from the organisms. These products may indicate the presence of a survival mechanism whereby mycobacteria might escape from the usual degradative processes.

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