INFECTION AND IMMUNITY, Feb. 1984, p. 531–535
0019-9567/84/020531-05$02.00/0

Vol. 43, No. 2

Macrophage Activation by Cord Factor (Trehalose 6,6′-Dimycolate): Enhanced Association with and Intracellular Killing of *Trypanosoma cruzi*

F. KIERSZENBAUM,* A. ZENIAN,† AND J. J. WIRTH

Department of Microbiology and Public Health, Michigan State University, East Lansing, Michigan 48824-1101

Received 19 September 1983/Accepted 6 November 1983

Cord factor (trehalose 6,6′-dimycolate[TDM]), a mixture of 6,6′-diesters of α,α-D-trehalose with natural mycolic acids, has been described as having immunoregulatory and antitumor activities in vivo, although the relevant mechanisms of action remain unelucidated. In this work, we measured the effects of TDM on both mouse macrophage association with (i.e., the combined result of surface binding and uptake) and subsequent intracellular killing of *Trypanosoma cruzi*, the causative agent of Chagas' disease. Pretreatment of macrophage cultures with TDM for 16 h markedly increased both the ability of these cells to associate with *T. cruzi* and the rate of killing of parasites. The results obtained with macrophages treated with TDM after exposure to the parasites did not differ from those obtained with untreated macrophages, indicating that macrophage activation did not occur immediately after TDM treatment and was time dependent. The TDM effect was reversible since the extents of macrophage-parasite association and intracellular killing returned to normal levels 4 h after TDM treatment. Neither catalase, which scavenges hydrogen peroxide, nor sodium azide or potassium cyanide, which are inhibitors of peroxidase activity, significantly reduced the level of trypanosome killing by TDM-treated macrophages. TDM also increased the uptake of glutaraldehyde-killed *T. cruzi* and latex particles, suggesting that TDM could act mostly by enhancing phagocytosis and that increased cell association with the living trypanosomes did not necessarily depend on the macrophages becoming more susceptible to parasite invasion. These results indicate that TDM modulates macrophage function by augmenting both internalization and intracellular destruction. Hydrogen peroxide and peroxidase activity, postulated to be involved in phagocytic killing of *T. cruzi*, did not appear to be an absolute requirement for the killing of *T. cruzi* in TDM-treated macrophages.

Macrophages play a dual role in infections caused by *Trypanosoma cruzi*, the unicellular parasite that causes Chagas' disease, because they not only contribute to host resistance mechanisms (3) but also can act as host cells for the parasite (2, 12). Although the requirements for uptake and destruction of this organism by phagocytic cells have received increasing attention in recent years (reviewed in reference 5; 10, 16, 17, 19, 20), mechanisms involved in the interaction remain largely unelucidated. The use of agents capable of modulating macrophage functions should facilitate exploration of the conditions and circumstances affecting macrophage interaction with *T. cruzi*. Of particular interest is cord factor (trehalose 6,6′-dimycolate [TDM]), a glycolipid product isolated from mycobacteria, corynebacteria, and several other microorganisms (reviewed in references 6 and 7), because it has been shown to stimulate the uptake of bacteria by peritoneal macrophages in vivo (11, 18), to stimulate certain macrophage functions (14), and, more recently, to induce an enhancement of host resistance against *T. cruzi* infection in mice (L. L. Leon, M. Q. Da Cruz, B. Galvao-Castro, G. H. Soares, A. Oliveira Lima, and E. Lederer, Fifth International Congress of Immunology, Kyoto, Japan). We show here that TDM treatment enhances the ability of macrophages to associate themselves with virulent forms of *T. cruzi* in vitro as well as increases the rate of intracellular destruction of the parasites by mechanisms which do not necessarily involve oxygen-dependent cytotoxicity.

* Corresponding author.
† Present address: Department of Pathology, University of California Medical Center, San Diego, CA 92103-9981.

MATERIALS AND METHODS

Macrophage monolayers. Peritoneal cells were obtained from unelicited 8- to 10-week-old Crl:CD-1 (ICR) BR Swiss mice (Charles River Breeding Laboratories, Portage, Mich.), as described in detail previously (19). The cell suspensions were adjusted to contain 10^6 cells taking up neutral red dye per ml in minimal essential medium with Hanks salts (MEM; GIBCO Laboratories, Grand Island, N.Y.) containing 1.3 g of sodium bicarbonate per liter and supplemented with 100 U of penicillin, 100 μg of streptomycin per ml, and 10% heat-inactivated fetal bovine serum (MEMS). Individual wells of Lab-Tek chamber slides (eight-well slides; Lab-Tek Products, Div. Miles Laboratories Inc., Naperville, Ill.) received 0.5 ml of the cell suspension and were incubated at 37°C in a moist atmosphere (5% CO_2 in air) for 6 h. Nonadherent cells were then removed by washing the monolayers several times with MEMS. The monolayers consisted of >95% nonspecific esterase-positive cells with typical macrophage morphology.

Parasites. *T. cruzi* Tulahuén was used in this work. Blood was drawn from the retro-orbital venous plexus of ether-anesthetized mice infected 13 to 16 days before with 10^5 parasites each by the intraperitoneal route. Circulating (trypomastigote) forms of the parasite were first purified by density gradient centrifugation over Lymphoprep (Nyegaard, Oslo, Norway) (1), followed by chromatography over a DEAE-cellulose column (9). The flagellates were concentrated by centrifugation at 800 × *g* for 10 min at 4°C, washed once with MEM supplemented with 0.2% bovine serum albumin (MEM-BSA; Sigma Chemical Co., St. Louis, Mo.), and resuspended in the same medium at 5×10^6 to 10×10^6

organisms per ml. Preparations of living parasites used in this work consisted of 99 to 100% viable organisms. In some experiments, killed *T. cruzi*, prepared by suspending *T. cruzi* in 2.5% glutaraldehyde at 20°C for 30 min and washing twice with MEM-BSA, were used.

TDM. Batches of purified TDM (6) used in this work were isolated from *Mycobacterium tuberculosis* Peurois and were generous gifts from E. Lederer (Laboratoire de Biochimie, Centre National de la Recherche Scientifique, Gif-sur-Yvette, France) and P. Lefrancier (Institut Choay, Montrouge, France). Sterile stock suspensions of TDM contained 0.5 mg/ml in MEM, dispersed by sonication as described previously (4). All dilutions were made in MEMS.

TDM treatment of *T. cruzi*. To establish whether *T. cruzi* trypomastigotes are sensitive to TDM, a suspension containing 2×10^6 organisms per ml in MEMS was incubated with either 5 μg of TDM per ml or medium alone at 37°C. Concentrations of motile parasites in these mixtures were determined at the end of a 24-h incubation period in a 5% CO_2 incubator.

TDM treatment of macrophages. TDM treatment was initiated immediately after the removal of nonadherent cells from peritoneal cell suspensions. The macrophage monolayers were incubated with 2.5 μg of TDM per ml of MEMS at 37°C for 4, 8, or 16 h in a 5% CO_2 incubator. The results of preliminary titrations showed this dose to be optimal for increasing macrophage association with *T. cruzi*. TDM was removed at the end of the incubation period by washing the adherent cell monolayers several times with MEM-BSA.

Assays of *T. cruzi* association with and intracellular killing by macrophages. Immediately after the removal of TDM from the cultures and the washings, 0.5 ml of trypanosome suspension containing 10^6 organisms was added, and the cultures were further incubated at 37°C for 90 min. Nonbound parasites were removed by washing the cultures three times with MEM-BSA. To measure cell-parasite association, which includes surface-bound as well as internalized trypanosomes, triplicate cell cultures were fixed with absolute methanol for 10 min immediately after the removal of free-swimming organisms. To measure the effect of TDM on intracellular killing of *T. cruzi*, fresh MEMS containing 2.5 μg of TDM per ml was added to separate sets of triplicate cultures, which were incubated for an additional 24 h and then fixed as described above. All cell monolayers were then stained with Giemsa, and not less than 200 randomly selected cells were examined microscopically (×1,000). Measurements made immediately after removing the nonbound parasites and at 24 h after their removal are referred to as obtained at 0 and 24 h, respectively. Parallel control assays in which the macrophages were treated with medium alone instead of TDM suspension were always included. In some experiments, sets of macrophage cultures subjected to treatment with either TDM or medium alone were incubated with living or glutaraldehyde-fixed flagellates, and the extent of cell-parasite association was determined. The mechanisms of cell-parasite interaction affected by TDM were explored by adding different concentrations of either catalase (Sigma) or metabolic inhibitors such as sodium azide or potassium cyanide to the cultures. These reagents were incorporated either together with or after the removal of the parasites and remained in the system until the termination of the experiment; their concentrations are given below. In all instances, the results are expressed as the number of parasites associated with 100 macrophages and the percentage of macrophages associated with one or more trypanosomes, in terms of the mean ± 1 standard deviation.

Presentation of results and statistical analysis. All results presented in the tables represent two or more separate experiments with identical protocol. Differences were considered to be significant if $P < 0.05$, as determined by the Mann-Whitney U test.

TABLE 1. Kinetics of the effect of TDM on the ability of mouse macrophages to associate with *T. cruzi*

TDM treatment	Time of treatment (h)[a]	No. of *T. cruzi* per 100 macrophages (mean ± SD)[b]	% of macrophages associated with *T. cruzi* (mean ± SD)[b]
No		26.3 ± 3.0	22.0 ± 0.2
Yes	4	26.8 ± 3.2 (1.8)	21.8 ± 1.0 (−0.8)
Yes	8	34.5 ± 1.6 (31.3)[c]	28.4 ± 0.9 (29.2)[c]
Yes	16	46.5 ± 4.2 (76.9)[c]	37.7 ± 1.3 (71.5)[c]

[a] Macrophages were pretreated with TDM for 4, 8, and 16 h before the addition of parasites (for details, see the text). The initial parasite to macrophage ratio in this experiment was 5:1.

[b] Values in parentheses are the percent change in macrophage-parasite association due to TDM pretreatment of macrophages.

[c] This value represents a statistically significant difference ($P < 0.05$) with respect to the corresponding control value.

TABLE 2. Comparison of the effects of treatment of macrophages with TDM before and after exposure to *T. cruzi* on intracellular killing of parasites

TDM treatment		Time (h)[a]	No. of *T. cruzi* per 100 macrophages (mean ± SD)[b]	% of macrophages associated with *T. cruzi* (mean ± SD)[b]
Before	After			
No		0	89.0 ± 8.0	47.1 ± 2.6
Yes		0	136.0 ± 9.0 (52.8)	64.3 ± 6.0 (36.5)
No	No	24	37.0 ± 5.0 (−58.4)	21.4 ± 3.3 (−54.6)
Yes	No	24	28.0 ± 3.0 (−79.4)	19.3 ± 3.4 (−70.0)
Yes	Yes	24	26.0 ± 7.0 (−80.9)	15.8 ± 3.6 (−75.4)
No	Yes	24	35.0 ± 7.0 (−60.7)	20.9 ± 5.5 (−55.6)

[a] Time of incubation of macrophages after the removal of the nonbound trypanosomes. The initial parasite to macrophage ratio in this experiment was 2.5:1. Macrophages were pretreated with TDM for 16 h before the addition of parasites (for details, see the text).

[b] Values in parentheses are the percent change in macrophage-parasite association. Positive values represent increased association measured at 0 time due to TDM pretreatment of macrophages. Negative values represent the extent of parasite killing that occurred during the 24-h incubation period after the removal of nonbound parasites and were calculated with reference to the corresponding control value obtained at 0 time. Unless otherwise indicated, values in parentheses represent a statistically significant difference ($P < 0.05$) with respect to the corresponding control values.

RESULTS

Effects of TDM on *T. cruzi* association with macrophages and subsequent intracellular parasite destruction. Pretreatment of macrophages with TDM caused a marked increase in their ability to associate (i.e., bind and internalize) with untreated trypanosomes (Table 1). Although a significant effect was obtained after incubating the macrophages with TDM for 8 h, a greater enhancement was noted after a 16-h incubation period. For this reason, macrophages were pretreated with TDM for 16 h in all subsequent experiments. TDM caused both an increase in the numbers of parasites per cell and an elevation in the percentage of infected macrophages (Table 2). When nonbound flagellates were

TABLE 3. Reversibility of the stimulatory effect of TDM on macrophage association with *T. cruzi*

Treatment of macrophages	Postincubation time (h)[a]	No. of *T. cruzi* per 100 macrophages (mean ± SD)[b]	% of macrophages associated with *T. cruzi* (mean ± SD)[b]
None	0	150.0 ± 2.0	63.4 ± 2.9
TDM	0	201.0 ± 3.0 (34.0)[c]	75.3 ± 2.2 (18.8)[c]
TDM	1	185.0 ± 3.0 (23.3)[c]	78.9 ± 1.3 (24.4)[c]
TDM	2	185.0 ± 2.0 (23.3)[c]	76.4 ± 0.3 (20.5)[c]
TDM	4	140.0 ± 3.0 (−6.7)	67.1 ± 4.3 (4.3)
TDM	6	141.0 ± 3.0 (−6.0)	68.0 ± 2.8 (7.3)

[a] Macrophages were incubated in fresh medium between the removal of TDM and the addition of parasites.

[b] Values in parentheses are the percent change with respect to the corresponding control value obtained at 0 time.

[c] The difference represented by this percentage was statistically significant ($P < 0.05$). Changes seen after 4 and 6 h were not statistically significant.

removed and the infected cells were incubated for an additional 24 h, the rate of intracellular killing was greater in macrophages that had been pretreated with TDM than in control macrophages treated with medium alone. The enhanced destructive capacity of TDM-treated macrophages was emphasized by the greater reduction in parasite load over a 24-h period, despite the fact that the number of organisms initially present in these cells was larger than that found in medium-treated macrophages. In this experiment, we also compared the effects of treating macrophages with TDM before and after exposing them to the parasites. Increased parasite destruction was not seen when macrophages were treated with TDM after being exposed to the flagellates. In experiments in which identical samples of a trypomastigote suspension were incubated with MEMS containing or lacking TDM (up to 5 μg/ml), measurements of parasite concentration taken after 24 and 48 h were comparable (data not shown).

Reversibility of the effect of TDM on macrophages. Macrophage cultures preincubated with TDM were washed and further incubated in fresh medium for various periods of time before being exposed to the parasites to establish whether the condition resulting in enhanced association with *T. cruzi* was long lasting. The results (Table 3) showed that TDM-treated macrophages behaved again as untreated macrophages 4 h after the removal of TDM from the cultures.

Possible mechanisms whereby TDM enhanced killing of *T. cruzi* by macrophages. The possibility that TDM increased macrophage uptake and destruction of *T. cruzi* by promoting production of intermediate products of the reduction of molecular oxygen was examined by using inhibitors of peroxidase activity, such as potassium cyanide and sodium azide, and catalase, a scavenger of hydrogen peroxide. The reduced ability of medium-treated macrophages to destroy trypanosomes over a 24-h period in the presence of either sodium azide or potassium cyanide was of borderline statistical significance (Table 4). Instead, these reagents were ineffective in altering the rate of parasite killing within TDM-treated macrophages, whether these reagents were added to the cultures after the removal of the parasites (Table 4) or at the time of addition of the parasites (data not shown). The addition of catalase had no effect on the abilities of medium- and TDM-treated macrophages to kill the parasites, even when the enzyme was present at concentrations much greater than those shown by other investigators to be effective in inhibiting killing of other microorganisms by phagocytes (13, 15) (Table 5). Although Table 5 shows results obtained with up to 4,000 U of catalase, essentially the same findings were made in three repeated experiments in which up to 32,000 U of the enzyme was used (data not shown).

Comparison of the effects of TDM on macrophage association with live and dead blood forms of *T. cruzi*. To establish whether TDM enhanced macrophage interaction with *T. cruzi* by stimulating phagocytosis or by rendering the macrophages more susceptible to penetration by the trypanosomes, experiments were conducted in which live and fixed trypomastigotes, as well as latex beads, were used in the macrophage-parasite association assay. The results (Table 6) revealed that the dead flagellates were more readily taken up by macrophages than were the living ones whether medium-treated or TDM-treated cells were used. Additionally, TDM enhanced the uptake of both dead organisms and latex particles.

DISCUSSION

These results show that TDM, which enhances host resistance against *T. cruzi* infection in the mouse model system, enhances the ability of macrophages to associate with (i.e., bind to and take up) and kill virulent blood forms of *T. cruzi*. This was documented by the significant increase in the number of parasites associated with TDM-treated macrophages and the greater decrease in the number of organisms remaining in these cells 24 h later with respect to the values obtained with macrophages treated with medium alone. The noted differences were consistently observed

TABLE 4. Inability of inhibitors of peroxidase activity to significantly alter the rate of killing of *T. cruzi* by TDM-treated macrophages

Treatment of macrophages		Time (h)[a]	No. of *T. cruzi* per 100 macrophages (mean ± SD)[b]	% of macrophages associated with *T. cruzi* (mean ± SD)[b]
Before	After			
None	None	0	117.0 ± 29.0	52.4 ± 10.0
TDM	None	0	195.0 ± 7.0 (66.7)	73.1 ± 3.1 (39.5)
None	None	24	58.0 ± 6.0 (−50.4)	28.7 ± 7.6 (−45.2)
None	Na_3N	24	72.0 ± 16.0 (−38.5)[c]	37.1 ± 8.7 (−29.2)[c]
None	KCN	24	88.0 ± 15.0 (−24.8)[c]	44.1 ± 6.5 (−15.8)[c]
TDM	None	24	57.0 ± 5.0 (−70.8)	34.1 ± 4.0 (−53.4)
TDM	Na_3N	24	59.0 ± 2.0 (−69.7)	32.3 ± 3.4 (−55.8)
TDM	KCN	24	71.0 ± 16.0 (−63.6)	38.2 ± 4.1 (−52.3)

[a] See Table 2, footnote *a*.

[b] See Table 2, footnote *b*.

[c] The difference represented by this percentage was not significantly significant.

TABLE 5. Inability to alter the rate of killing of *T. cruzi* by TDM-treated macrophages with catalase

Treatment of macrophages		Time (h)[a]	No. of *T. cruzi* per 100 macrophages (mean ± SD)[b]	% of macrophages associated with *T. cruzi* (mean ± SD)[b]
Before	After			
None	None	0	72.6 ± 13.0	47.8 ± 6.7
TDM	None	0	123.0 ± 19.0 (69.4)	63.6 ± 6.3 (33.1)
None	None	24	34.1 ± 3.7 (−53.0)	24.5 ± 4.9 (−48.7)
None	Catalase[c]	24	32.4 ± 1.5 (−55.4)	16.7 ± 3.1 (−34.9)
None	Catalase[d]	24	41.0 ± 5.4 (−43.5)	22.6 ± 7.3 (−52.7)
None	Catalase[e]	24	23.5 ± 3.8 (−67.6)	17.0 ± 2.9 (−64.4)
TDM	None	24	26.8 ± 3.7 (−78.2)	19.2 ± 3.1 (−69.8)
TDM	Catalase[c]	24	32.4 ± 1.0 (−73.7)	18.7 ± 3.0 (−70.6)
TDM	Catalase[d]	24	34.0 ± 6.0 (−72.4)	20.3 ± 2.7 (−68.1)
TDM	Catalase[e]	24	31.8 ± 5.3 (−74.1)	19.6 ± 2.1 (−69.2)

[a] See Table 2, footnote *a*.
[b] See Table 2, footnote *b*.
[c] The concentration of catalase in the culture was 1,000 U/ml.
[d] The concentration of catalase in the culture was 2,000 U/ml.
[e] The concentration of catalase in the culture was 4,000 U/ml.

whether the average number of trypanosomes per 100 macrophages or the percentage of macrophages associated with the parasites was measured (Tables 1 and 2). The stimulatory effect of TDM was clearly exerted during the 16-h pretreatment period since additional exposure of the cultures to TDM during the subsequent 24-h incubation period failed to increase the rate of intracellular killing of parasites. Furthermore, when pretreatment was omitted and TDM was present during the 24 h after the removal of the nonbound trypanosomes, the results were comparable to those obtained with macrophages treated with medium alone (Table 2). This observation also indicated that once TDM had exerted its stimulatory effect, persistence of the stimulus was not necessary for macrophages to produce greater parasite uptake and killing. In addition, the lack of enhancing effect of TDM added to the cultures after the parasites had been removed suggested that the phagocytic cells required several hours of exposure to TDM (probably ca. 16 h) to display their activated state. Macrophages were reversibly activated by TDM since their ability to both associate with and kill *T. cruzi* significantly declined with time after incubation in fresh medium, returning to normal levels 4 h after treatment with TDM (Table 3).

Killing of *T. cruzi* by phagocytic cells has been postulated to involve peroxidase activity (15) or production of hydrogen proxide (10) or both. In our experimental system, parasite killing by medium-treated macrophages was slightly inhibited by potassium cyanide or sodium azide (Table 4), indicating a certain extent of involvement of peroxidase activity in the cytotoxic mechanism. These inhibitors seemed to have no major effect on TDM-enhanced killing of *T cruzi* since they failed to significantly reduce parasite killing by TDM-treated macrophages. Furthermore, decomposition of hydrogen peroxide with catalase was of little consequence since a very small change or no change in TDM-enhanced killing of parasites occurred in the presence of relatively large concentrations of this enzyme (Table 5). These results are in keeping with those of Lepoivre et al. (8), who found that in vitro production of hydrogen peroxide by mouse macrophages was elevated by TDM only after pretreatment of the cells with phorbol myristate acetate. Based on the above results, it appears either that peroxidase activity and hydrogen peroxide are not significantly involved in intracellular killing of *T. cruzi* by TDM-treated macrophages or that in the absence of these oxygen-dependent processes alternative cytotoxic mechanisms may take over.

Finally, the facts that TDM also enhanced phagocytosis of dead parasites and that the fixed organisms were more readily taken up than the living ones (Table 6) suggest the unlikelihood that TDM renders macrophages more susceptible to trypanosome invasion. Therefore, a major effect of TDM could be to stimulate phagocytosis. Alternatively, it could be inferred from the relatively rapid decay in the TDM-induced effects on phagocytosis once TDM is removed that the actual presence of TDM on the macrophage surface enhanced binding and phagocytosis. The rapid turnover of macrophage membrane components might bring about the loss of surface-bound TDM. Additional work is required to explain the intriguing observation of greater uptake of killed trypanosomes, which implies an ability of the living flagellates to partially elude phagocytosis.

Collectively, the present results highlight the capacity of TDM to induce macrophage activation in terms of increased association with and subsequent killing of an intracellular parasite. The noted effects of TDM on macrophage activity

TABLE 6. Comparison of the effects of TDM on macrophage association with live and dead *T. cruzi* and with latex particles

Macrophage interaction with[a]:	TDM treatment[b]	No. of *T. cruzi* per 100 macrophages (mean ± SD)[c]	% of macrophages associated with *T. cruzi* or latex (mean ± SD)[c]
Live *T. cruzi*	No	83.0 ± 2.4	51.5 ± 2.4
	Yes	131.8 ± 2.5 (58.8)	67.9 ± 1.1 (32.8)
Dead *T. cruzi*	No	166.3 ± 11.4	72.6 ± 1.3
	Yes	266.6 ± 8.0 (60.3)	84.1 ± 1.2 (15.8)
Latex beads	No	ND[d]	66.1 ± 0.6
	Yes	ND	80.9 ± 3.6 (22.4)

[a] The initial parasite to macrophage ratios were 5:1 and 2.5:1 for live and dead parasites, respectively.
[b] Macrophages were pretreated with TDM for 16 h.
[c] Values in parentheses are the percent change in macrophage-parasite (or -latex particle) association seen after the removal of nonbound parasites (0 time). All of these values represent statistically significant differences ($P < 0.05$) between results obtained with and without TDM.
[d] ND, Not done.

may be relevant to the mechanisms whereby the bacterial product enhances host defense and causes immunological potentiation.

ACKNOWLEDGMENTS

We thank E. Lederer and P. Lefrancier for supplying the TDM used in this study.

This work was supported by Public Health Service grants AI 14848 and AI 17041 from the National Institutes of Health.

LITERATURE CITED

1. **Budzko, D. B., and F. Kierszenbaum.** 1974. Isolation of *Trypanosoma cruzi* from blood. J. Parasitol. **60**:1037–1038.
2. **Dias, E.** 1932. Le *Trypanosoma cruzi* et ses rapports avec le système reticuloendothélial. C. R. Séanc. Soc. Biol. **106**:206–210.
3. **Kierszenbaum, F., E. Knecht, D. B. Budzko, and M. C. Pizzimenti.** 1974. Phagocytosis: a defense mechanism against infection with *Trypanosoma cruzi*. J. Immunol. **112**:1839–1844.
4. **Kierszenbaum, F., and D. R. Walz.** 1981. Proliferative responses of central and peripheral rat lymphocytes elicited by cord factor (trehalose 6,6′-dimycolate). Infect. Immun. **33**:115–119.
5. **Kuhn, R. E.** 1981. Immunology of *Trypanosoma cruzi* infections, p. 137–165. *In* J. M. Mansfield (ed.), Parasitic diseases, vol. 1. Marcel Dekker, Inc., New York.
6. **Lederer, E.** 1976. Cord factor and related trehalose esters. Chem. Phys. Lipids **16**:91–106.
7. **Lederer, E.** 1979. Cord factor and related synthetic trehalose diesters. Springer Semin. Immunopathol. **2**:133–148.
8. **Lepoivre, M., J.-P. Tenu, G. Lemaire, and J.-F. Petit.** 1982. Antitumor activity and hydrogen peroxide release by macrophages elicited by trehalose diesters. J. Immunol. **129**:860–866.
9. **Mercado, T. I., and K. Katusha.** 1979. Isolation of *Trypanosoma cruzi* from blood of infected mice by column chromatography. Prep. Biochem. **9**:97–106.
10. **Nathan, C., N. Nogueira, C. Juangbhanich, J. Ellis, and Z. Cohn.** Activation of macrophages *in vivo* and *in vitro*. Correlation between hydrogen peroxide and killing of *Trypanosoma cruzi*. J. Exp. Med. **149**:1056–1068.
11. **Parant, M., F. Parant, L. Chedid, J.-C. Drapier, J.-F. Petit, J. Wietzerbin, and E. Lederer.** 1977. Enhancement of non-specific immunity to bacterial infection by cord factor (trehalose 6,6′-dimycolate). J. Infect. Dis. **135**:771–777.
12. **Pizzi, T.** 1957. Inmunologia de la enfermedad de Chagas, p. 152–157. Universidad de Chile, Santiago.
13. **Sasada, M., and R. B. Johnston, Jr.** 1980. Macrophage microbicidal activity. Correlation between phagocytosis-associated oxidative metabolism and the killing of *Candida* by macrophages. J. Exp. Med. **152**:85–98.
14. **Tenu, J.-P., E. Lederer, and J.-F. Petit.** 1980. Stimulation of thymocyte mitogenic protein secretion and of cytostatic activity of mouse peritoneal macrophages by trehalose 6,6′-dimycolate and muramyl-dipeptide. Eur. J. Immunol. **10**:647–653.
15. **Villalta, F., and F. Kierszenbaum.** 1983. Role of polymorphonuclear cells in Chagas' disease. I. Uptake and mechanisms of destruction of intracellular forms of *Trypanosoma cruzi* by human neutrophils. J. Immunol. **131**:1504–1510.
16. **Williams, D. M., and J. S. Remington.** 1977. Effect of human monocytes and macrophages on *Trypanosoma cruzi*. Immunology **32**:19–23.
17. **Wirth, J. J., and F. Kierszenbaum.** 1982. Inhibitory action of elevated levels of adenosine-3′:5′-cyclic monophosphate on phagocytosis: effects on macrophage-*Trypanosoma cruzi* interaction. J. Immunol. **129**:2759–2762.
18. **Yarkoni, E., and A. Bekierkunst.** 1976. Nonspecific resistance against infection with *Salmonella typhi* and *Salmonella typhimurium* induced in mice by cord factor (trehalose-6,6′-dimycolate) and its analogues. Infect. Immun. **14**:1125–1129.
19. **Zenian, A., and F. Kierszenbaum.** 1981. Inhibition of macrophage-*Trypanosoma cruzi* interaction by concanavalin A and differential binding of bloodstream and culture forms to the macrophage surface. J. Parasitol. **68**:408–415.
20. **Zenian, A., and F. Kierszenbaum.** 1983. *Trypanosoma cruzi*: differences in cell surface interaction of circulating (trypomastigote) and culture (epimastigote) forms with macrophages. J. Parasitol. **69**:660–665.