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Roles of the Fc Receptor and Respiratory Burst in Killing of *Rickettsia prowazekii* by Macrophagelike Cell Lines

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It is known that the virulent strain of *Rickettsia prowazekii* grows in macrophagelike cell lines, but if the rickettsiae are treated with antirickettsial serum before infection, the intracellular rickettsiae fail to grow and are destroyed. The uptake of rickettsiae by macrophagelike cell lines was increased by treatment of the rickettsiae with immune serum and with purified immunoglobulin G (IgG) from this serum but not by treatment with the $F(ab')_2$ fragment derived from this IgG. This suggested that the normal rickettsial pathway of entry could be augmented by the Fc receptor-mediated pathway. However, rickettsiae treated with these $F(ab')_2$ fragments which contained no Fc region were destroyed as effectively as those treated with immune serum or IgG. Internalization of *R. prowazekii* (whether virulent, avirulent, treated, or untreated) did not lead to an increased release of CO_2 from [1-^{14}C]glucose, an increase that would have been indicative of a respiratory burst. Furthermore, a mutant macrophagelike cell line, incapable of a respiratory burst, was able to destroy rickettsiae treated with immune serum as effectively as did the parental cell line. Electron micrographs of macrophagelike cells which had been incubated with either antirickettsial IgG or with $F(ab')_2$ fragments derived from this IgG both demonstrated marked deterioration of the rickettsiae, which were primarily within vacuoles but occasionally free in the cytoplasm. In contrast, untreated rickettsiae displayed morphologically normal rickettsiae which were mostly in the cytoplasm but occasionally in the intact and damaged vacuoles. These results indicated that (i) a respiratory burst was not a significant part of the mechanism used by macrophagelike cells to destroy *R. prowazekii* treated with immune serum, (ii) the destruction of the rickettsiae by the macrophage was not dependent on a diversion to the Fc receptor-mediated pathway of entry, and (iii) the locus of damage to the rickettsiae was most likely the phagolysosome of the macrophagelike cell line.

Rickettsia prowazekii, the etiological agent of epidemic typhus in humans, is an obligate intracellular bacterium which grows in host cell cytoplasm. The virulent Breinl strain of *R. prowazekii* is capable of growing in both macrophage and nonmacrophage cells, but the growth of the avirulent Madrid E strain is inhibited in macrophage cells and macrophagelike cell lines (5, 7, 10). However, the growth of virulent rickettsiae which have been treated with specific antisera prior to infection is inhibited in macrophage cells and macrophagelike cell lines (1, 5, 6, 10). In contrast, specific antiserum-treated rickettsiae of either strain grow normally in fibroblasts (10, 17), indicating that the combination of the antiserum and a macrophage-specific factor is necessary for antirickettsial activity. Macrophagelike cells are used since they are similar to human macrophages in their interaction with *R. prowazekii*, are clonal and subject to less heterogeneity, and undergo a respiratory burst and since a mutant cell line exists that has lost this ability.

R. prowazekii is thought to enter a fibroblastic host cell by inducing its own phagocytosis in association with a phospholipase A activity (13, 15). Macrophage and macrophagelike cells carry Fc receptor on their surface (9), so it seems reasonable that antibody-coated rickettsiae are phagocytized by the Fc receptor-mediated pathway instead of by, or in addition to, the induced phagocytosis pathway. Accordingly, the antirickettsial activity in Fc receptor-bearing cells would be dependent on the pathway used by the rickettsiae for entry into the host cell. If this hypothesis were correct, the antirickettsial activity should be dependent on the Fc domain of the antibody molecule. In this study, macrophagelike cell lines were infected with rickettsiae that had been treated with normal serum, antirickettsial serum, immunoglobulin G (IgG) from this serum, or the $F(ab')_2$ fragment of the antirickettsial antibodies, and the growth of the rickettsiae in these cells was compared.

The nature of the antibody-induced, antirickettsial mechanism in macrophagelike cells is unknown. One of the mechanisms by which these cells can destroy invading bacteria is the respiratory burst, in which, following phagocytosis, there is increased activity of the hexose monophosphate (HMP) shunt, elevated oxygen consumption, and production of superoxide radicals and hydrogen peroxide (7). In order to determine whether the respiratory burst plays a role in the antibody-dependent killing of rickettsiae in macrophagelike cells, two approaches were taken. First, the respiratory burst was measured in macrophagelike cells infected with rickettsiae and either untreated or treated with specific IgG. The second approach was to choose a mutant macrophagelike cell line which lacks the ability to activate the respiratory burst (3), infect it with IgG-treated Breinl strain, and observe whether it displayed an antirickettsial activity similar to that of the parental cell line.

MATERIALS AND METHODS

Rickettsiae and cell cultures. *R. prowazekii* Breinl and Madrid E were grown in embryonated eggs, purified, and stored as described previously (10). The concentration of viable rickettsiae was enumerated by their hemolytic activity

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(11). The mouse macrophagelike cell line RAW264.7 was obtained from the Cell Distribution Center at the Salk Institute, San Diego, Calif., and grown in Dulbecco modified Eagle medium supplemented with 10% newborn bovine serum. The mouse macrophagelike cell lines J774.1, J774.2, J774.16, and J774.C3C were provided by B. R. Bloom (Albert Einstein College of Medicine, Bronx, N.Y.) and grown in Dulbecco modified Eagle medium supplemented with nonessential amino acids and 10% horse serum. The mouse fibroblast cell line L929 was grown in Eagle minimal essential medium supplemented with 10% calf serum. All sera were heat inactivated at 56°C for 30 min before use so that complement did not influence these studies. All cells were grown in a humidified atmosphere of 3% CO_2 in air at 34°C.

Antiserum IgG and F(ab')$_2$. Antiserum was prepared in a rabbit immunized with rickettsiae in adjuvant as previously described (12). Antiserum was heat inactivated (56°C, 30 min) and stored at −80°C. The antiserum had a titer of 1:10^6 as determined by indirect enzyme-linked immunosorbent assay. IgG was purified from the antiserum by means of a protein A column kit, and F(ab')$_2$ fragments were purified from IgG fractions by using immobilized pepsin and protein A columns according to the directions of the manufacturer (Pierce Chemical Co., Rockford, Ill.). The concentrations of IgG and F(ab')$_2$ fractions were determined by their A_{279} (2). The F(ab')$_2$ preparation had undetectable IgG contamination by both enzyme-linked immunosorbent assay and sodium dodecyl sulfate-polyacrylamide gel electrophoresis methods.

Infection of cells with rickettsiae. X-irradiated as well as non-X-irradiated RAW264.7 cells were used. J774 and L929 cells were not X irradiated prior to infection. Irradiation was performed as previously described (10) to inhibit the growth of the host cell and to make the measurement of rickettsial growth parameters easier. X-irradiated RAW264.7 cells were seeded in eight-chambered slides (7.5×10^4 cells per chamber), 35-mm-diameter plates (3×10^5 cells per plate), or 24-well plates (5×10^4 to 10×10^4 cells per well). Nonirradiated cells were seeded 24 or 48 h before infection at 2.5×10^4 to 5.0×10^4 cells per cm^2 of growth area.

Antibody solutions were added to rickettsial suspensions at 0.2 to 4.5 μg per 10^6 viable rickettsiae (10^6 to 10^7 rickettsiae per ml). The suspensions were incubated prior to infection for 15 min at room temperature with occasional gentle stirring and then were diluted threefold with Hanks balanced salt solution supplemented with 0.1% gelatin and 5.0 mM L-glutamic acid–monopotassium salt to obtain the final volume and concentration needed for infection.

Two modes of infection, static and centrifugal, were used. In the static mode, rickettsiae (multiplicity of infection, 160 to 200) were added to washed cells for 1 h, and then the cells were washed twice and incubated with fresh medium at 34°C in a CO_2 incubator. The centrifugal mode of infection enables one to efficiently infect cells with low amounts of rickettsiae (multiplicities of infection, 5 to 15 viable rickettsiae per cell). A monolayer of cells in a 24-well plate containing the rickettsial suspensions (0.3 ml per well) was centrifuged for 15 min at 500 × *g* at room temperature, incubated for 45 min at 34°C, washed, and incubated with 0.5 ml of medium per well at 34°C in a CO_2 incubator.

Rickettsial counts. At zero time, i.e., immediately after the 1-h infection period, and at the indicated times postinfection, cells from chamber slides or cover slips from the wells were dried, fixed, and stained by a modification of the Gimenez method as described by Wisseman et al. (16). All treatments were done in duplicates at each time. Cells were examined microscopically at ×1,000 magnification, and the number of rickettsiae in each of 100 cells was determined. When there were more than 50 rickettsiae per cell, a value of 50 was assigned. This caused an underestimation at later times with heavy growth. The percentage of infected cells and the average number of rickettsiae per infected cell were determined. The number of rickettsiae per chamber, plate, or well was calculated as follows: %R × IR × number of cells per vessel, where %R is the percentage of infected cells and IR is the average number of rickettsiae per cell. The ratio of the number of rickettsiae at a given time to the number of rickettsiae at zero time was referred to as the extent of rickettsial growth.

Specific immunofluorescence staining was used to demonstrate IgG or F(ab')$_2$ on the treated rickettsiae in the infected cells at zero time. In all experiments, the rickettsiae treated with specific antibodies [antiserum, IgG, or F(ab')$_2$] were always fluorescent, whereas the untreated or the normal serum-treated rickettsiae were not.

Determination of the HMP shunt activity. The measurement of glucose oxidation via the HMP shunt is based on the ability of the cell to oxidize [1-^{14}C]glucose in preference to [6-^{14}C]glucose (4, 8). This assay measures the basis of the respiratory burst; unless the shunt is activated, there can be no burst. Because of the interaction of live rickettsiae with superoxide and peroxide, this is a more reliable assay for the burst than the measurement of the products. Cells were seeded at 3×10^6 to 4×10^6 cells per 25-cm^2 flask containing 0.4 mM glucose. Prior to infection, cells were washed once with a solution of Dulbecco phosphate-buffered saline supplemented with 0.1% gelatin, 5.0 mM glutamic acid monopotassium salt, 0.4 mM glucose, and 15.9 μg of phenol red per ml. The cells were infected in the static mode (or mock infected) with rickettsial suspensions diluted in the supplemented Dulbecco phosphate-buffered saline solution (3 ml per flask). The ability of mock-infected cells to undergo a respiratory burst induced by phorbol 12-myristate 13-acetate (PMA) or by opsonized zymosan was confirmed.

Immediately following infection, the flasks were labeled with [1-^{14}C]glucose (55.6 mCi/mmol; Amersham Corp., Arlington Heights, Ill.) or [6-^{14}C]glucose (58.5 mCi/mmol; Amersham) at 0.5 μCi per flask, and the flasks with CO_2 traps were sealed. The CO_2 traps consisted of 3-ml plastic Pasteur pipettes (Saint-Amand Mfg. Co., San Fernando, Calif.) with "windows" cut in their bulb ends. CO_2 evolution was determined by standard methods. Cells from unlabeled flasks were used to determine the rickettsial growth parameters at the end of the incubation.

Electron microscopy. RAW264.7 cells were infected by the centrifugal method with *R. prowazekii* Breinl [untreated or treated with 2 μg of antirickettsial IgG or F(ab')$_2$ fragments derived from this IgG per 10^6 rickettsiae, at a multiplicity of infection of 15]. After this 1-h period, the cells were fixed in 3.0% glutaraldehyde in 66 mM cacodylate buffer (pH 6.8) containing 2.5 mM $CaCl_2$. Preembedding in 2% Difco agar was followed by (i) brief rinse with buffer, (ii) postfixation in 1% osmium tetroxide buffered with 66 mM cacodylate buffer for 1 h at 4°C, (iii) brief rinse in distilled water, and (iv) dehydration through graded methanol. During the dehydration steps, blocks were stained for 1 h at room temperature with 0.5% uranyl acetate in 30% methanol. All blocks were embedded in Spurr epoxy resin. Ultrathin sections were stained with either 0.5% uranyl acetate in 50% ethanol and lead citrate or potassium permanganate and were examined in a Jeol CX100 electron microscope operated at 80 kV. Duplicate samples from two experiments were coded, and

TABLE 1. Initial infection values in RAW264.7 cells infected with *R. prowazekii*

Type of cells[a]	Treatment[b]	No. of expts	Avg ± SE	
			%R[c]	No. of IR[d]
X irradiated	No Ab	7	83 ± 5	4.4 ± 0.3
	NRS	3	78 ± 5	5.4 ± 0.7
	ARS	3	89 ± 2	31.3 ± 5.2
	IgG	7	88 ± 3	26.5 ± 3.0
	$F(ab')_2$	6	70 ± 4	4.0 ± 0.3
Nonirradiated	No Ab	2	53 ± 5	1.8 ± 0.1
	IgG	2	73 ± 1	5.4 ± 0.7
	$F(ab')_2$	2	49 ± 2	1.7 ± 0.2

[a] Cells were irradiated with a 2,500-rad dose, seeded in eight-chamber slides (7.5×10^4 cells per chamber), and infected with *R. prowazekii* Breinl (multiplicity of infection, 200; static mode). Nonirradiated cells were seeded in 35-mm-diameter plates, grown for 2 days, and infected at a concentration of 2.5×10^5 to 3.0×10^5 per plate with *R. prowazekii* Breinl (multiplicity of infection, 160; static mode).

[b] Normal rickettsial serum (NRS) was used at a concentration of 2.7 μg/10^6 rickettsiae, equivalent to the higher concentration of antirickettsial serum (ARS). Antirickettsial serum and IgG were used over a range of 0.2 to 2.7 μg/10^6 rickettsiae. $F(ab')_2$ was used at 0.5 to 1.3 μg/10^6 rickettsiae. Ab, Antibody.

[c] %R, Percentage of infected cells.

[d] IR, Average number of rickettsiae per infected cell.

the electron micrographs were interpreted without knowledge of the treatment that the rickettsiae had received.

RESULTS

Initial infection of RAW264.7 cells. The RAW264.7 macrophagelike cells were infected more extensively by Breinl strain rickettsiae which were treated with specific IgG (antirickettsial serum or purified IgG) than by untreated rickettsiae (Table 1). The percentage of the cells infected by treated rickettsiae was only slightly elevated in comparison to that infected by control rickettsiae, but the average number of rickettsiae per infected cell was six- to sevenfold higher in the X-irradiated cells and threefold higher in the nonirradiated cells than in the controls. In contrast, the initial infections by normal serum-treated and antirickettsial $F(ab')_2$ fragment-treated rickettsiae were similar to that by the untreated rickettsiae (Table 1). The results suggested that the Fc receptors on the macrophagelike cells did take part in the phagocytosis of the IgG-coated rickettsiae by providing an additional pathway of entry and provided evidence (in addition to that achieved by enzyme-linked immunosorbent assay and sodium dodecyl sulfate-polyacrylamide gel electrophoresis methods) that the $F(ab')_2$ preparation did not contain a significant amount of IgG.

Effect of specific antiserum, purified IgG, and $F(ab')_2$ fragments on the growth of rickettsiae. All preparations of antirickettsial antibodies [antiserum, purified IgG, and $F(ab')_2$ fragments] had an inhibitory effect on the growth of the virulent rickettsiae in RAW264.7 cells (Table 2). The concentration of antibodies which caused inhibition of rickettsial growth in the RAW264.7 cells was in the range of 0.2 to 2.7 μg/10^6 viable rickettsiae. Lower concentrations did not have any inhibitory effect on the growth of the rickettsiae. Similarly, in J774 cells infected by the more efficient centrifugal mode of infection, in which the infections with and without IgG were similar, marked inhibition of rickettsial growth was observed for the rickettsiae treated with either antirickettsial IgG or $F(ab')_2$ fragments (Table 2). The control rickettsiae grew 35- to 50-fold in this period, while the number of the IgG or $F(ab')_2$-treated rickettsiae increased only 5-fold. These results supported the contention that the Fc fragment of the antibody molecule was not needed for the inhibition of rickettsial growth.

The rickettsiae in all protocols, untreated as well as IgG or $F(ab')_2$ treated, grew in the mouse fibroblast cell line L929 to

TABLE 2. Effect of specific antibodies on growth of *R. prowazekii* Breinl in macrophagelike and nonmacrophage cell lines

Cell line[a]	Day	MOI[b]	Mode[c]	Result[d] after treatment with[e]:				
				Control (ratio)	IgG		$F(ab')_2$	
					Ratio	%C	Ratio	%C
L929	1	5	C	5.2 ± 2.6 (2)	4.9 ± 2.3 (2)	94	4.1 ± 1.4 (2)	79
L929	2	5	C	22.1 ± 10.3 (2)	23.8 ± 9.0 (2)	108	26.2 ± 5.5 (2)	119
RAW264.7(X)	1	200	S	4.0 ± 0.4 (5)	0.8 ± 0.1 (3)	20	1.4 ± 0.3 (5)	35
RAW264.7(X)	2	200	S	7.1 ± 0.5 (7)	0.7 ± 0.1 (6)	10	1.8 ± 0.1 (5)	25
RAW264.7	2	160	S	34.7 ± 4.3 (2)	1.7 ± 0.4 (2)	5	5.2 ± 0.3 (2)	15
J774.1	2	5	C	34.7 ± 1.6 (2)	6.0 ± 1.4 (2)	17	3.4 ± 1.1 (2)	10
J774.2	2	5	C	49.5 ± 17.0 (2)	4.8 ± 2.5 (2)	10	5.1 ± 2.8 (2)	10
J774.16	1	10	C	5.1 ± 6.8 (2)	0.4 ± 0.1 (2)	8		
J774.16	2	10	C	16.1 ± 5.6 (2)	1.1 ± 0.9 (2)	7		
J774.C3C	1	10	C	4.9 ± 1.3 (2)	0.8 ± 0.3 (2)	16		
J774.C3C	2	10	C	17.6 ± 3.3 (2)	1.0 ± 0.9 (2)	6		

[a] RAW264.7 X-irradiated cells (X) were infected in eight-chamber slides at a concentration of 7.5×10^4 cells per chamber. Nonirradiated RAW264.7 cells were grown in 35-mm-diameter plates and infected at a concentration of 2.5×10^5 to 3.0×10^5 per plate. All J774 cells were grown in 24-well plates and were infected at a concentration of 5×10^4 to 10×10^4 cells per well. The L929 cells were grown in 24-well plates and were infected at a concentration of 2×10^4 to 3×10^4 cells per well.

[b] MOI, Multiplicity of infection.

[c] C and S, Centrifugal and static modes of infection, respectively.

[d] Ratio of the number of rickettsiae at a given time to the number of rickettsiae at zero time.

[e] The antibody/rickettsiae ratios used were 0.2 to 2.7 μg of IgG and 1.0 to 1.6 μg of $F(ab')_2$ per 10^6 viable rickettsiae in RAW264.7 cell experiments and 4.5 μg of IgG and 3.0 μg of $F(ab')_2$ in L929 and J774 cell experiments. Numbers in parentheses indicate the number of determinants. %C, Percent control.

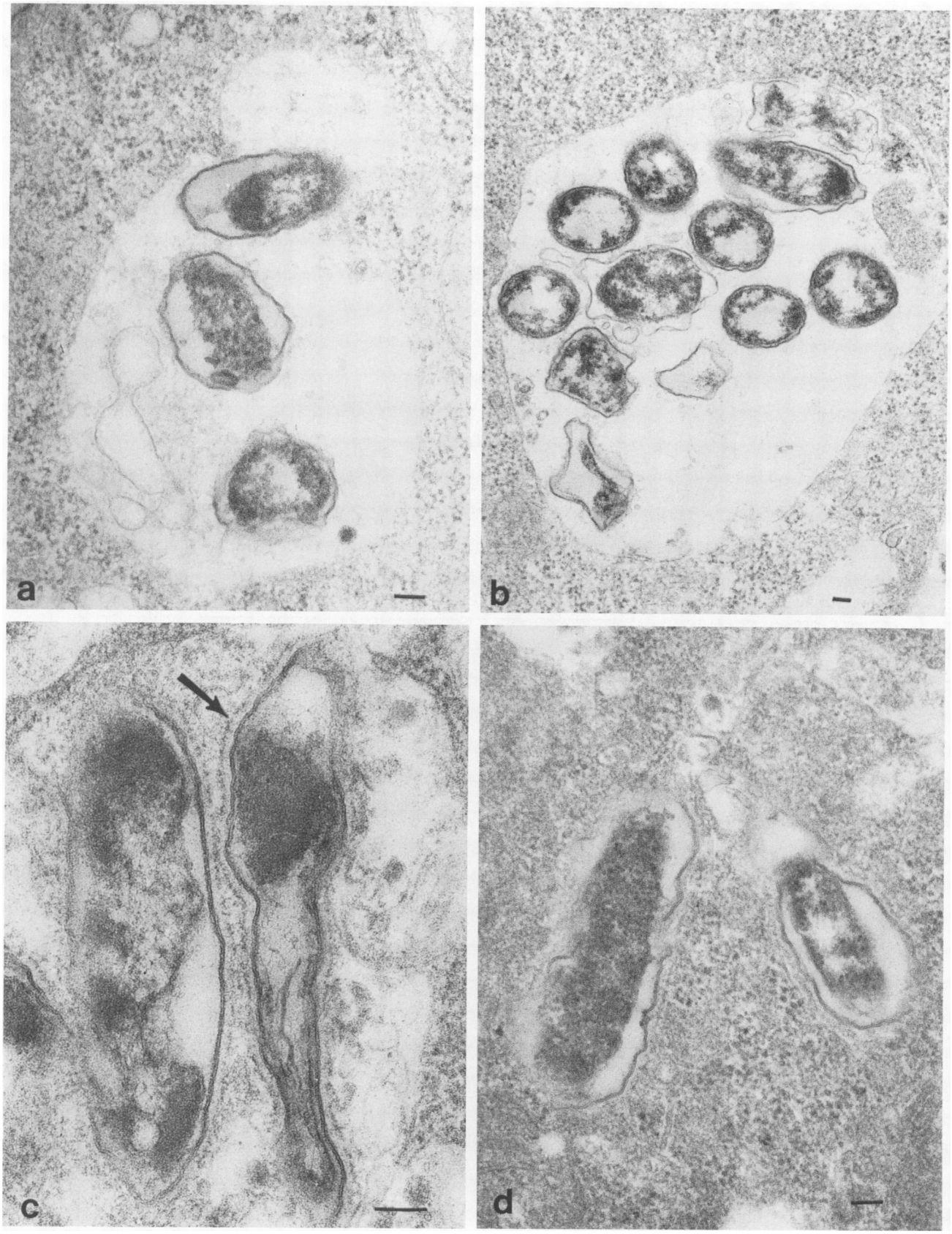

FIG. 1. Transmission electron micrographs of thin sections of *R. prowazekii* Breinl in mouse macrophagelike cell line RAW264.7. (a, c, and d) Treatment of rickettsiae with anti-*R. prowazekii* IgG; (b) treatment of rickettsiae with anti-*R. prowazekii* $F(ab')_2$ fragments (derived from anti-*R. prowazekii* IgG). Note the presence of exopolysaccharide resulting from the interaction of anti-*R. prowazekii* IgG with this layer (arrow). Bar = 100 nm.

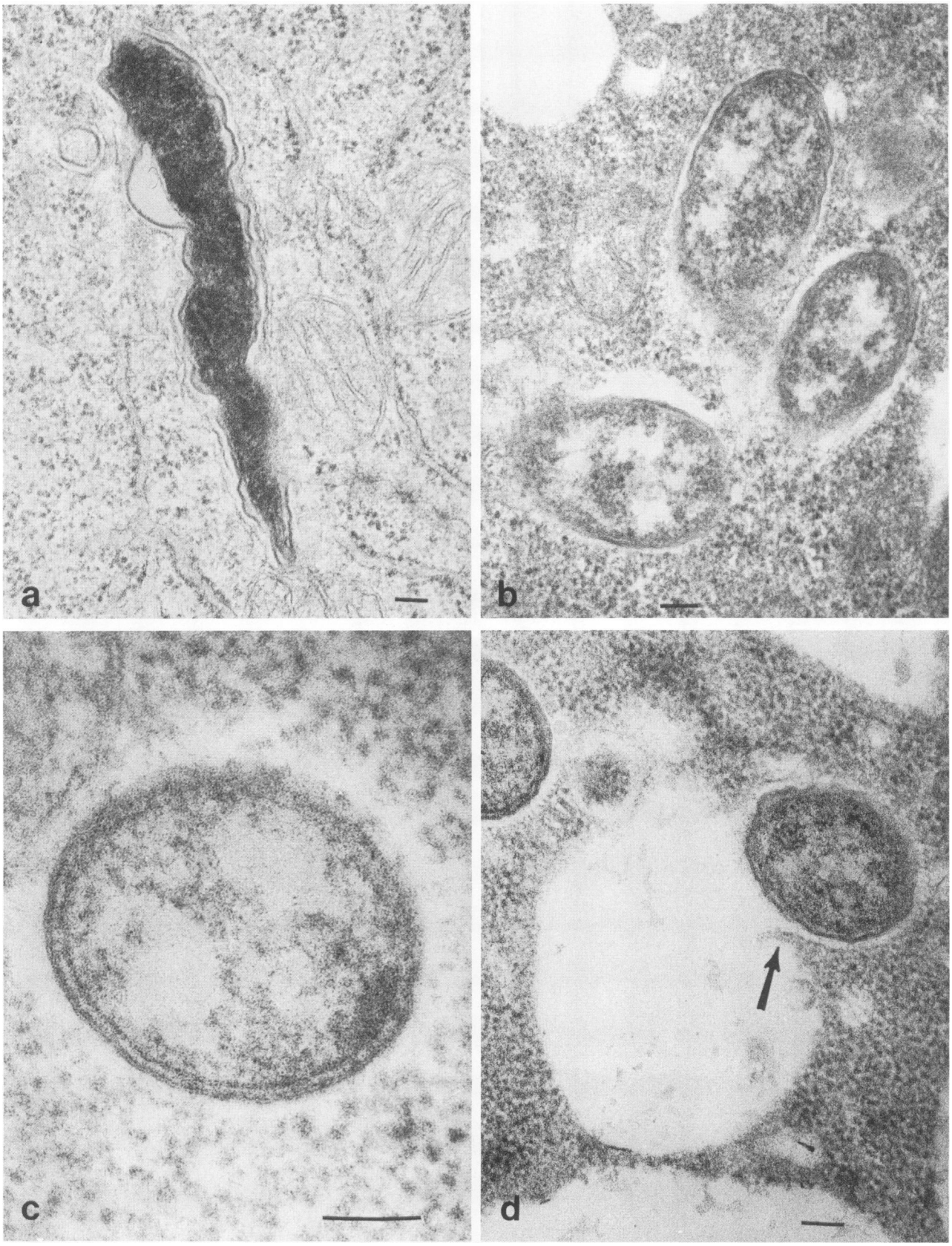

FIG. 2. Transmission electron micrographs of thin sections of *R. prowazekii* Breinl in mouse macrophagelike cell line RAW264.7. (a) Treatment of rickettsiae with anti-*R. prowazekii* $F(ab')_2$ fragments (derived from anti-*R. prowazekii* IgG). (b, c, and d) Treatment with normal serum. (c) Normal appearing rickettsia residing in cytoplasm. Note the absence of vacuolar membrane surrounding the rickettsia. (d) Fragmentation of the vacuolar membrane (arrow) caused by penetration of the rickettsia into the cytoplasm. Bar = 100 nm.

TABLE 3. HMP shunt activity in RAW264.7 cells infected with *R. prowazekii*

Treatment[a]	*n*	CO_2 evolution from [1-^{14}C]glucose (pmol [10^6] of cells/min)
No Rp	12	21 ± 6
PMA	8	192 ± 38
RpB	10	28 ± 11
RpB + IgG	8	27 ± 11
RpE	4	20 ± 3

[a] Rickettsial infection values were in the range of 2.7 to 3.7 rickettsiae per cell in untreated *R. prowazekii* Breinl, 9.0 to 17.7 in IgG-treated *R. prowazekii* Breinl, and 4.1 to 5.3 in untreated *R. prowazekii* Madrid E. Monitoring rickettsial growth at 1 or 2 days postinfection showed inhibition of rickettsial growth in the *R. proazekii* Madrid E- and IgG-treated *R. prowazekii* Breinl-infected cells only. Rp, *R. prowazekii*; RpB, *R. prowazekii* Breinl; RpE, *R. prowazekii* Madrid E.

the same extent. No inhibition of growth by antirickettsial antibodies was observed in nonmacrophage cells.

Examination by electron microscopy. Electron micrographs of macrophagelike cells which had been incubated with either antirickettsial IgG or $F(ab')_2$ fragments derived from this IgG both demonstrated marked deterioration of the rickettsiae which were primarily within vacuoles (Fig. 1a and b). The affected rickettsiae showed convolution of the outer membrane, widening of the periplasmic space, aggregation of the ribonucleoprotein materials, and loss of morphological integrity of the nucleoid region. The interaction of the antirickettsial IgG with the rickettsiae revealed, by potassium permanganate staining, an electron-dense fibrous layer, the exopolysaccharide, extending from the outer membrane of the rickettsiae (Fig. 1c). An electron-lucent layer separated this electron-dense fibrous layer from the outer membrane. Occasionally, the affected rickettsiae were free in the cytoplasm (Fig. 1d and 2a). The location of such rickettsiae was recognized by the absence of any vacuolar membrane surrounding the rickettsiae. In contrast, micrographs of untreated rickettsiae displayed morphologically normal rickettsiae, mostly in the cytoplasm (Fig. 2b). An absence of vacuolar membrane surrounding the rickettsiae signified their free cytoplasmic existence (Fig. 2c). Occasionally, the rickettsiae were found in intact and in damaged vacuoles (Fig. 2d). Such vacuoles showed some fragmentation of the vacuolar membranes as the rickettsiae penetrated into the cytoplasm (Fig. 2d). Samples were then taken from parallel cultures for light micrographic determinations of rickettsial growth; they demonstrated killing of rickettsiae treated with either IgG or $F(ab')_2$, as described above. In agreement with our results, Meyer reported that *R. prowazekii* organisms treated with antirickettsial serum were found in phagolysosomes and that they had abnormal morphology, suggesting that they were no longer viable (W. A. Meyer III, Ph.D. thesis, University of Maryland, Baltimore, 1982).

Role of the respiratory burst. The HMP shunt activity monitored by the release of CO_2 from [1-^{14}C]glucose in the RAW264.7 macrophagelike cells served as a criterion for the respiratory oxidative burst. It was confirmed in preliminary experiments that the amount of CO_2 produced from [6-^{14}C]glucose by the cells was only 10% of the amount produced from the [1-^{14}C]glucose, and it did not increase when cells were induced with PMA. The HMP shunt activity of RAW264.7 cells did not increase significantly when cells were infected with either the virulent or the attenuated strain of rickettsiae or with IgG-treated virulent rickettsiae (Table 3). However, the cells in the same experiments displayed their capacity to undergo oxidative burst when induced with PMA by an 11-fold increase in their HMP shunt activity. Experiments with the J774.1 macrophage cell line showed no increase in HMP shunt activity when cells were infected in the same way with the various rickettsiae (data not shown).

The lack of association of the respiratory burst with the killing of the rickettsiae was confirmed by using a mutant cell line, J774.C3C, which lacks the ability to undergo the respiratory burst, probably due to a defect in a *b*-type cytochrome (3). We confirmed that PMA-treated J774.C3C cells failed to produce a burst (data not shown). In both the mutant and parental (J774.16) cell lines, the growth of the rickettsiae that had been treated with the antirickettsial IgG was inhibited, while the extent of growth in the untreated rickettsiae increased 16-fold after 2 days (Table 2).

DISCUSSION

The macrophagelike cells engulfed several times more rickettsiae that had been treated with antirickettsial serum or with IgG than untreated, normal serum-treated, or $F(ab')_2$-treated rickettsiae. However, the inhibition of the growth of rickettsiae treated with antirickettsial $F(ab')_2$ fragments in RAW264.7 and J774 macrophagelike cell lines was similar to that induced by antirickettsial serum and IgG treatment. No inhibition was seen with normal serum or normal IgG or in fibroblasts. The antirickettsial activity appeared to be macrophage cell specific but Fc receptor independent, so that redirection of rickettsiae from induced phagocytosis to Fc receptor-mediated phagocytosis failed as a hypothesis to explain rickettsial killing by the macrophage.

Two well-known mechanisms can be used in the macrophage cell to deal with microbial infection: oxygen dependent and oxygen independent. The oxygen-dependent mechanism, the respiratory burst, was undetectable in these cells when either the avirulent or virulent rickettsiae, whether opsonized with antirickettsial serum or not, were internalized. Furthermore, in a mutant macrophagelike cell line which lacks the ability to undergo the respiratory burst, inhibition of growth of IgG-treated rickettsiae was comparable to that in the parental, wild-type cells. Meyer reported that the interaction between human monocyte-derived macrophages and Breinl strain rickettsiae treated with human immune or nonimmune serum did not result in luminol-dependent chemiluminescence activity and that the monocyte-derived macrophage of chronic granulomatous disease patients (which is unable to undergo respiratory burst) inhibited the growth of immune serum-treated rickettsiae (Meyer, Ph.D. thesis). These results provided evidence that the respiratory burst mechanism was not responsible for the antirickettsial activity within the macrophagelike cells.

It had been found that the fusion of L929 cells containing *R. prowazekii* free in the cytoplasm with macrophagelike cells led to the preferential elimination of Madrid E strain rather than Breinl strain in the heterokaryon (14). This result demonstrates that the macrophagelike cell has the capacity to destroy intracytoplasmic rickettsiae. However, the presence of damaged rickettsiae within vacuoles (presumably phagolysosomes) when the rickettsiae had been treated with either antirickettsial IgG or $F(ab')_2$ suggested that antibody inhibited a component on the rickettsia which was necessary for the rapid escape of the rickettsia from the phagosome prior to fusion with lysosomes. It was notable that some treated rickettsiae, although damaged, were found free in the cytoplasm. The nature of the rickettsial activity that would be inhibited and the macrophage lysosomal activity that

would be necessary for rickettsial killing in accordance with this hypothesis remains to be established.

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