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Processing of Streptococcal Cell Walls by Rat Macrophages and Human Monocytes In Vitro

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Phagocytosis and degradation of cell walls by peritoneal macrophages obtained from Fischer 344 or Buffalo rats was measured in tissue culture. Group A cell wall antigen, detected by immunofluorescence, persisted in cultured rat macrophages for at least 40 days, whereas group D cell wall material was eliminated by 6 to 8 days. This same pattern of persistence of group A cell walls and elimination of group D cell walls was observed in cultures of human monocytes followed for 24 days in culture. Group A streptococcal cell walls labeled with either [^{14}C]alanine or [^{14}C]glucose were handled in a similar manner by macrophages from either Fischer 344 or Buffalo rats. In contrast, [^{14}C]glucose-labeled group D cell walls were degraded at a much faster rate. Buffalo macrophages were more efficient than Fischer 344 macrophages in degrading group D cell walls. The inability of macrophages to degrade group A cell walls was not due to a failure of lysosomes to fuse with phagosomes. Neither serum lysozyme in the culture medium nor cell wall-associated autolysin contributed to the degradation of group D cell walls by macrophages. Neither immune serum nor macrophages obtained from specifically immunized rats influenced phagocytosis or persistence of group A cell walls.

Several experimental models of chronic granulomatous inflammation can be produced by the injection of group A streptococcal cell wall fragments. Chronic remittent multinodular lesions develop in rabbit skin after a single intradermal injection of cell walls (10, 15). Rheumatic-like heart lesions are produced in mice (4, 11) and chronic polyarthritis is produced in rats by a single intraperitoneal injection (14; W. J. Cromartie, J. G. Craddock, and J. H. Schwab, Fed. Proc., p. 477, 1968). In each of these models, chronic inflammation is associated with the localization and persistence of cell wall antigens. The material responsible for the induction and perpetuation of chronic lesions is the mucopeptide–C polysaccharide complex of the cell wall (14). The persistence of cell wall fragments from group A streptococci and certain other bacteria is postulated to play a role in the pathogenesis of chronic diseases that develop subsequent to bacterial infection (14). Cell walls from group D streptococci are rapidly eliminated from tissue and do not induce chronic or recurrent lesions (16). For this reason group D cell walls have been used as a control material in animals and are used in the present study as an example of a biodegradable bacterial cell wall.

In experimental models a consistent finding is the localization of cell wall material within macrophages in the lesions. This indicates that the macrophage has an essential role in the distribution and persistence of bacterial components. It is important, therefore, to examine in detail the interaction of streptococcal cell walls and macrophages; i.e., to determine (i) what the macrophage does to the cell wall structures and (ii) how the ingested cell wall affects macrophage functions. As an experimental approach to these problems we have studied rat peritoneal macrophages in tissue culture. This is part of a study designed to establish the mechanism by which poorly degradable group A streptococcal cell walls induce chronic inflammatory disease in the periodontal tissue and joints of rats and to define the nature of genetic control of susceptibility in these experimental diseases.

A few studies have described the processing of radiolabeled bacteria after phagocytosis in vitro (1, 3, 20) or incubation with leukocyte extracts (6–8). However, only Gallis et al. (6) have followed the fate of an identifiable cell structure, and most studies were limited to a few hours of culture. The present work follows the fate of phagocytized radiolabeled streptococcal cell walls in macrophages cultured in vitro from 1 to 6 weeks. The phagocytized cell wall structures are identified by immunofluorescent techniques as well as radioisotopes. In this paper the

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ingestion and processing of cell wall fragments by cultured macrophages is compared in peritoneal cells obtained from Fischer 344 rats, which are susceptible to streptococcal-induced polyarthritis, and Buffalo rats, which do not develop these joint lesions (S. K. Anderle and W. J. Cromartie, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, B49, p. 19). An accompanying paper compares the activation of macrophages from these rat strains to become cytotoxic for target cells (19).

MATERIALS AND METHODS

Macrophages. We define macrophages as adherent, mononuclear cells that actively phagocytize particles. Peritoneal macrophages were obtained from normal or thioglycolate-stimulated female Buffalo or Fischer 344 rats (Simonsen Laboratories, Inc., Gilroy, Calif.) weighing 100 to 200 g. Five days after intraperitoneal injection of 7 ml of fluid thioglycolate broth (Baltimore Biological Laboratory, Cockeysville, Md.), the peritoneum was washed three times with cold Hanks balanced salt solution containing 10 U of preservative-free heparin sodium (The Upjohn Co., Kalamazoo, Mich.), 100 mg of streptomycin sulfate, and 100 U of penicillin G per ml. After washing the peritoneal cells twice in medium 199 (Grand Island Biological Co., Grand Island, N.Y.) supplemented with 10% heat-inactivated horse serum and antibiotics, 2×10^6 to 4×10^6 cells were added to tissue culture dishes (10 by 35 mm; Falcon Plastics, Oxnard, Calif.) or sterile glass cover slips (22 by 22 mm). After 2 h of incubation at 37°C in 5% CO_2 the medium was changed, and cultures were incubated for 18 h. Before addition of streptococcal cell walls, macrophage cultures were washed three times with medium 199 to remove nonadherent and nonviable cells. Cultures contained greater than 95% adherent macrophages, based on morphology of Giemsa-stained slides and phagocytosis of polystyrene latex particles (The Dow Chemical Co., Midland, Mich.). Continued adherence of cells containing cell wall material was considered evidence that the macrophages remained viable during the culture period. Trypan blue staining was also done on representative cultures at intervals up to 40 days.

Human peripheral blood monocytes, band 1 cells from Ficoll-Hypaque gradients, were supplied by John Spitznagel. These were washed twice in TC 199 containing 10% heat-inactivated fetal calf serum and antibiotics. Monocytes (10^6) were cultured on glass cover slips at 37°C in 5% CO_2 and 100% humidity. After 24 h, cultures were washed three times with TC 199, and fresh medium with serum and antibiotics was added.

Radiolabeled streptococci. Group A streptococcal cells (type 3, strain D58) were labeled with either [^{14}C]alanine or [^{14}C]glucose. Cells were cultured for 24 h in a modified medium consisting of 1 volume of "depleted broth" (filtered, spent medium of an 18-h Todd-Hewitt broth culture of group A streptococci) and 1 volume of "glucose-salts" medium modified from that of Shockman et al. (17). To this medium was added 1 μCi of DL-[1-^{14}C]alanine per ml (specific activity, 23.8 mCi/mmol; Amersham/Searle, Arlington Heights, Ill.).

Labeled glucose was incorporated into cells by culturing them for 6 h in freshly prepared beef heart infusion broth, in which the only glucose added was D-[*U*-^{14}C]glucose, 1.5 μCi/ml (specific activity, 180 mCi/mmol; ICN Canada Ltd., Montreal, Quebec, Canada). After 6 h the culture was supplemented with glucose-salts medium (17) and incubated for an additional 12 h. Group D (strain F-24) streptococcal cells were labeled with [^{14}C]glucose in a similar procedure except that the total incubation was 12 h, to preclude autolysis of these organisms.

Preparation of streptococcal cell wall fragments. The preparation of unlabeled streptococcal cell wall fragments from group A (strain D58) and group D (strain F-24) streptococci has been described (10). Cell wall fragments from radiolabeled group A and group D streptococci were prepared according to the procedure described by Salton and Horne (13), using a Mickle cell disintegrator with glass beads. After disruption of cells, cell wall fragments were collected by differential centrifugation followed by treatment with trypsin and ribonuclease (10). Cell wall preparations were exhaustively washed with phosphate-buffered saline to remove unincorporated radioactivity and subsequently stored at −20°C. Rhamnose content of cell wall fragments was determined by the method of Dische and Shettles (5).

Distribution of radiolabel in cell wall fragments. The distribution and identification of radiolabel incorporated into the cell walls of group A streptococci was determined by paper chromatography (9). Acid-hydrolyzed labeled cell wall fragments were chromatographed on Whatman no. 1 filter paper (Whatman, Inc., Clifton, N.J.) by ascending chromatography, with *n*-butanol–acetic acid–water (60:20:10) as the first solvent and a water-saturated phenol solution with an ammonium atmosphere as the second solvent. Amino acids and amino sugars were developed with ninhydrin spray (E. Merck AG, Darmstadt, Germany), and sugars were developed with aniline phthalate spray (E. Merck). Reference amino acids and sugars were chromatographed individually and in mixture to determine their distribution. Two chromatograms for each labeled cell wall preparation were run simultaneously. One was developed, whereas coincident areas from the undeveloped chromatogram were cut, placed in scintillation fluid, and counted in a liquid scintillation counter. The amount of radioactivity in a given area of a chromatogram was compared to the amount of radioactivity added initially and expressed as the percent distribution of radiolabel in cell walls.

Phagocytosis and fate of cell walls. The fate of phagocytized streptococcal cell walls was followed by immunofluorescent and radioisotope techniques. A qualitative estimate of the persistence of phagocytized cell walls in macrophages was accomplished by determining the number of macrophages in a microscope field that displayed fluorescence, and the intensity of this fluorescence, after direct staining with fluorescein-conjugated rabbit antistreptococcal antibodies (10). Quantitative measurement of the phagocytosis and degradation of streptococcal cell walls by cultured macrophages was done with radiolabeled cell walls. At various times after the addition of labeled cell walls to macrophage cultures, replicate cultures were washed

three times with phosphate-buffered saline and digested with 0.1 N NaOH for 30 min. Portions were then taken for protein determination according to the method of Oyama and Eagle (12) to determine the relative number of macrophages remaining in culture. The remaining cell digest was placed on GF-82 glass fiber paper (W. and R. Balston Ltd., London, England), air dried, placed in Omnifluor-toluene (New England Nuclear, Boston, Mass.), and counted in a Packard liquid scintillation counter (Packard Instrument Co., Inc., Rockville, Md.). Degradation of streptococcal cell walls by macrophages was determined by measuring the amount of radioactivity that passes through a 0.22-μm membrane filter (Millipore Corp., Bedford, Mass.). Portions of medium, washes, and NaOH digests of macrophage cultures that had phagocytized labeled cell walls were filtered, and the radioactivity that passed through the filter was degraded cell wall material, whereas retained material was considered nondegraded. Evidence that ^{14}C that passes through the filter represents degraded cell wall is provided by the control cell wall suspensions, not fed to macrophages, in which the filterable ^{14}C was always less than 4%. A positive control was provided by group D cell wall label, which does pass through the filter as it is degraded.

Immunofluorescence was used in addition to the quantitative radioisotope technique, because merely following ^{14}C gives no indication of the nature of the labeled material. Conjugated antibody at least demonstrates that the ingested cell wall retains structures that can bind antibody.

Immunization of rats. Buffalo and Fischer 344 rats were immunized with heat-killed vaccines of group A (strain D58) streptococci by injection into the tail vein twice weekly for 3 weeks. Rats were "sensitized" by subcutaneous injection into the hind footpads and back with a viable group A streptococcal vaccine in complete Freund adjuvant as described by Bültmann et al. (2).

Fluorescein-conjugated rabbit antiserum. Rabbits were hyperimmunized with group A streptococcal vaccine or group D cell walls to obtain antibodies against cell wall antigens. The immunization procedure, absorption of serum, and fluorescein conjugation of the antibodies have been described (10).

RESULTS

Distribution of radiolabel in streptococcal cell walls. The incorporation and distribution of [^{14}C]alanine and [^{14}C]glucose into group A streptococcal cell walls with culture conditions described in Materials and Methods are shown in Table 1. Although only about 3 to 5% of the radiolabel was incorporated into whole cells, greater than 50% of incorporated radioactivity was associated with the cell wall fragments. DL-[1-^{14}C]alanine is the more specific label, since 85% of the radioactivity in cell walls from streptococci cultured in the presence of this amino acid had label associated with alanine after acid hydrolysis and chromatography. In cells of streptococci cultured in the presence of [^{14}C]glucose, about 75% of the label incorporated into cell walls was equally distributed between rhamnose, glucose, and glucosamine.

Phagocytosis. Table 2 compares the phagocytic capacity of macrophages obtained from Fischer 344 and Buffalo rats. The kinetics of phagocytosis were almost identical between macrophages obtained from the two rat strains. No significant difference in the phagocytic indexes of macrophages from these rats was observed whether the macrophages were obtained from normal or thioglycolate-stimulated animals. The presence of immune serum has been reported to influence the phagocytic process (3); however, in the present study, the addition of 10% anti-group A serum to macrophage cultures did not affect phagocytosis. Similarly, macrophages from rats immunized with a viable group A streptococcal vaccine (2) phagocytized labeled cell walls to the same extent as normal macrophages.

Direct demonstration of the phagocytosis of streptococcal cell walls by cultured macrophages was obtained by fluorescent staining. Immunofluorescent reactive material was found

TABLE 1. *Incorporation and distribution of DL-[1-^{14}C]alanine and D-[U-^{14}C]glucose into group A streptococcal cell walls*

Radiolabel	Incorporation into cell walls (%)[a]	Sp act (cpm/μg of rhamnose) (× 10^3)	Distribution (%)[b]
DL-[1-^{14}C]alanine	53.8	1.4	85 (Alanine)
D-[U-^{14}C]glucose	80.3	2.9	25 (Rhamnose) 24 (Glucosamine) 24 (Glucose)

[a] (Counts per minute in cell walls/counts per minute in whole cells) × 100 = percent incorporation of radiolabel into cell walls.

[b] (Counts per minute in chromatographic area/total counts per minute added to chromatogram) × 100 = percent distribution of radiolabel.

TABLE 2. *Comparison of phagocytosis of group A cell walls by thioglycolate-stimulated Fischer 344 and Buffalo macrophages*

Period of phagocytosis (h)	Phagocytic index[a] (cpm/μg of protein)	
	Fischer 344	Buffalo
1	6.9 ± 0.69	6.4 ± 0.69
3	24.6 ± 1.48	25.2 ± 0.41
6	44.3 ± 2.66	44.3 ± 5.3
9	52.7 ± 2.88	57.0 ± 3.15
12	60.1 ± 0.57	69.6 ± 8.15

[a] Mean phagocytic index of three cultures, ± standard deviation.

sequestered throughout the macrophage cytoplasm, giving a brilliant granular fluorescent pattern (Fig. 1).

Persistence of phagocytized cell walls in rat macrophages and human monocytes. After the incubation of streptococcal cell walls with cultured macrophages for 24 h, the nonphagocytized cell walls were removed by washing cultures with medium 199. At various intervals thereafter cultures were assayed for the persistence or loss of phagocytized material by immunofluorescent staining or scintillation counting. Culture media were changed every 4 days. Greater than 90% of the macrophages that remained adherent to culture vessels or slides after washing with saline were found to be viable by trypan blue exclusion.

Group D streptococcal cell walls were completely eliminated by Fischer 344 and Buffalo macrophages 6 to 8 days after phagocytosis, as determined by the absence of immunofluorescent reactive material in macrophages (Fig. 1). In contrast, immunofluorescent reactive material persisted in macrophages containing group A streptococcal cell walls for up to 40 days. All of the macrophages contained cell wall material that retained the capacity to bind fluorescein-conjugated antibody specific for group A cell wall polysaccharide. The intensities of the fluorescence were similar in macrophages from both rat strains.

The fact that group A cell walls are not eliminated in this system could be due to the failure of fusion of lysosomes with phagosomes. To test this, group A and group D cell wall fragments were added simultaneously to macrophage cultures. After 24 h the nonphagocytized cell walls were removed, and cultures were followed for up to 31 days. Table 3 shows the fate of phagocytized group A and group D cell walls in thioglycolate-stimulated Fischer macrophages. Group D cell walls could not be detected by 6 to 9 days. Group A cell walls in the same macrophages, however, persisted for up to 31 days, with no significant loss of reactivity with conjugated antiserum.

The phagocytosis and fate of group A and group D streptococcal cell walls in human monocytes mimics that of rat macrophages. Table 4 summarizes three experiments on the persistence of streptococcal cell walls in human monocytes. As with rat macrophages, human monocytes eliminated group D cell walls by 6 days, whereas group A cell walls persisted for up to 24 days, as demonstrated by reaction with conjugated antiserum (Fig. 1).

The fate of radiolabeled group A cell walls is shown in Table 5. There was an initial loss of 22 to 43% of the cell wall material during the first 2 days of culture. This was due to the death of adherent cells, which were subsequently washed off the culture plates. Thereafter, the protein content of cultures, which reflects the number of viable adherent cells, and the ^{14}C-labeled group A cell walls remained constant. This is consistent with the immunofluorescent results. In contrast, the amount of ^{14}C-labeled group D cell walls remaining with the adherent cells showed a continuous decline over this period of observation which was greater than the rate of decline of viable adherent cells (Table 6).

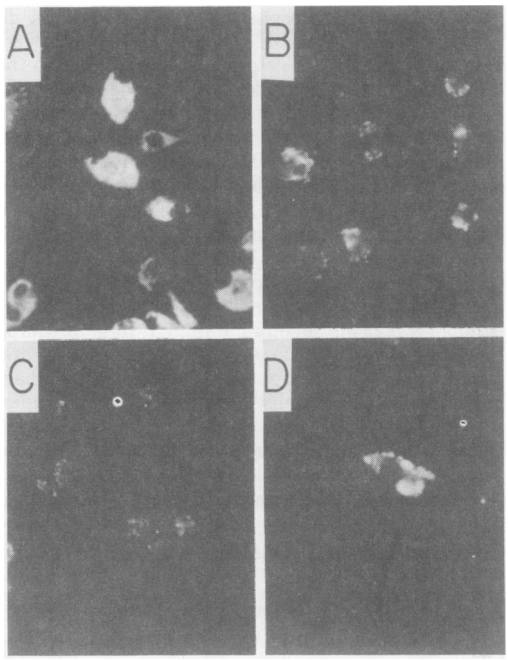

FIG. 1. *Immunofluorescent staining of phagocytized streptococcal cell walls in cultures of rat macrophages or human monocytes. (A) Fischer macrophages 40 days after in vitro phagocytosis of group A cell walls. Anti-group A polysaccharide conjugate, ×440. (B) Fischer macrophages 24 h after phagocytosis of group D cell walls. Anti-group D conjugate, ×440. (C) As in (B), but 4 days after phagocytosis of group D cell walls. (D) Human monocytes 24 days after phagocytosis of group A cell walls. Anti-group A polysaccharide conjugate, ×440. Control macrophage cultures, not given cell walls, displayed only a diffuse pattern of autofluorescence when stained with fluorescein-conjugated antibodies.*

Degradation of phagocytized cell walls. Although the above studies show that group A cell wall structures can persist in viable macrophages in culture, it is possible that degradation is proceeding without elimination of the cell wall fragments. To examine this, we used filterability as a measure of cell wall breakdown. The

radioactivity that passed through a 0.22-μm Millipore membrane filter was defined as degraded cell wall material. Less than 4% of the radioactivity of the original labeled cell wall was filterable, and this did not increase after treatment with 0.1 N NaOH. In all cultures, 95% or more of the radioactivity initially phagocytized could be accounted for in the medium, washes, and macrophages from a given culture.

Less than 4% of the phagocytized group A streptococcal cell walls that remained intracellular in normal macrophages from either rat strain was degraded after 8 days in culture (Table 5). At this time, 10 to 16% of the radioactivity released into the culture medium from macrophages was degraded. Although this might suggest that some slow degradation of group A cell walls has occurred, this is of doubtful significance because only a small fraction (0 to 10%) of the ^{14}C label within adherent cells appears extracellular. Cell walls labeled with [^{14}C]glucose were degraded by macrophages from both rat strains to a slightly greater extent than [^{14}C]alanine-labeled cell walls.

In contrast to group A cell walls, up to 83% of the group D cell walls within macrophages was degraded after 8 days in culture (Table 6). The Fischer 344 macrophages retained a greater amount of intracellular degraded cell wall material. The extracellular medium of macrophage cultures from normal and thioglycolate-stimulated Buffalo rats contained significantly more degraded cell wall material, compared with Fischer 344 macrophage cultures (Table 6). This was observed in a number of experiments and is the only consistent difference between these rat strains in the degradative capacities of macrophages. Macrophage protein increased after 8 days in culture after ingestion of group A cell walls (Table 5). This is because protein synthesis is stimulated in activated macrophages. Protein did not increase after ingestion of group D cell walls (Table 6), consistent with the failure to maintain an activated state in macrophages.

Heat inactivation of the autolysin associated with radiolabeled group D cell walls (18) and absorption of serum lysozyme with bentonite did not effect degradation.

The persistence of radiolabeled group A streptococcal cell walls in macrophages was found to be essentially the same in cultures containing either normal or immune serum. Furthermore, macrophages obtained from immunized rats in the presence or absence of nonadherent cells (lymphocytes) exhibited similar patterns of cell wall persistence.

TABLE 3. *Fate of simultaneously phagocytized group A and group D cell walls in thioglycolate-stimulated Fischer macrophages*

Fluorescein-conjugated antiserum	Days after removal of nonphagocytized cell walls[a]	Intensity of fluorescence
Anti-A	1	4+
	2	4+
	4	4+
	6	4+
	9	4+
	11	4+
	31	4+
Anti-D	1	4+
	2	4+
	4	2+
	6	±
	9	−

[a] A mixture of 50 μg of group A cell walls per ml and 100 μg of group D cell walls per ml was added to macrophage cultures simultaneously.

TABLE 4. *Fate of phagocytized group A and group D cell walls in human monocytes*[a]

Cell walls phagocytized	Days after removal of nonphagocytized cell walls	Intensity of fluorescence
Group A, 50 μg/ml	4	4+
	6	4+
	12	4+
	16	4+
	24	4+
Group D, 100 μg/ml	2	3+
	4	1+
	6	±
	10	−

[a] Monocytes were cultured in TC 199 with 10% heat-inactivated fetal calf serum. Duplicate cover slip cultures were stained with fluorescein-conjugated antibody specific for group A or group D cell wall antigens.

DISCUSSION

The degradation of isotopically labeled bacteria after phagocytosis by cells in culture has been studied by several investigators (1, 3, 20). Cohn (3) found that the rate of degradation of bacterial lipids, nucleic acids, and proteins by phagocytic cells is primarily dependent upon the composition of the ingested organism and, particularly, the composition of the bacterial surface or cell wall. Spector et al. (20), using bacteria iodinated with ^{125}I to study the fate of granuloma-inducing bacteria phagocytized by cultured macrophages, found that by 72 h 95% of group A streptococci were degraded to mate-

TABLE 5. *Comparison of the fates of group A cell walls in unstimulated Fischer 344 and Buffalo macrophages*

Macrophages (rat strain)	Radiolabeled cell walls	Period of culture (days)[a]	% Protein remaining[b] (mean ± SD)	% ^{14}C remaining[c] (mean ± SD)	Degradation (%)[d]	
					Intracellular	Extracellular
Fischer 344	[^{14}C]alanine	2	78.4 ± 7.02	78.4 ± 5.72	2.2 ± 0.20	3.4 ± 0.15
		4	59.2 ± 3.69	74.1 ± 6.19	2.2 ± 0.20	5.9 ± 0.26
		8	89.9 ± 5.25	72.7 ± 11.8	3.3 ± 0.62	10.7 ± 0.40
Fischer 344	[^{14}C]glucose	2	62.1 ± 7.56	75.8 ± 14.7	2.7 ± 0.15	7.7 ± 0.20
		4	54.9 ± 2.48	63.3 ± 7.41	3.1 ± 0.25	11.3 ± 0.47
		8	60.5 ± 4.59	63.8 ± 14.2	3.9 ± 0.62	16.2 ± 0.79
Buffalo	[^{14}C]alanine	2	61.4 ± 1.39	71.1 ± 5.97	2.2 ± 0.15	3.8 ± 0.05
		4	63.6 ± 6.51	67.2 ± 5.44	1.9 ± 0.23	6.0 ± 0.26
		8	92.9 ± 5.17	74.5 ± 3.20	3.0 ± 0.11	11.8 ± 0.86
Buffalo	[^{14}C]glucose	2	60.8 ± 3.20	56.9 ± 8.14	3.0 ± 0.32	6.5 ± 0.32
		4	58.7 ± 15.7	55.4 ± 4.38	2.9 ± 0.28	9.1 ± 0.05
		8	71.1 ± 4.48	57.2 ± 8.53	3.7 ± 0.40	14.4 ± 0.32

[a] Period of culture after a 24-h challenge with 40 μg of [^{14}C]alanine- or 20 μg of [^{14}C]glucose-labeled group A cell walls per culture. Cultures were washed to remove nonphagocytized cell walls after 24 h.

[b] Measure of viable adherent cells. Protein content at given time compared to cultures before cell walls were added. SD, Standard deviation.

[c] Calculated as percentage of radioactivity in macrophages at given time as compared with that present after the intial 24-h phagocytic period. Three determinations. SD, Standard deviation.

[d] Percent degradation: intracellular, fraction of radioactivity within macrophages that is filterable; extracellular, fraction of radioactivity in culture medium that is filterable. Mean (± standard deviation) of three determinations.

TABLE 6. *Comparison of the fates of group D cell walls labeled with [^{14}C]glucose in Fischer 344 and Buffalo normal and stimulated macrophages*[a]

Macrophages[b] (rat strain)	Period of culture (days)	% Protein remaining (mean ± SD)	% ^{14}C remaining (mean ± SD)	Degradation (%)	
				Intracellular	Extracellular
Fischer 344 (normal)	2	80.6 ± 6.85	79.3 ± 8.70	57.8 ± 4.73	16.2 ± 1.51
	4	53.8 ± 7.27	47.5 ± 4.34	75.4 ± 0.10	33.4 ± 1.15
	8	41.4 ± 7.26	31.2 ± 2.94	83.5 ± 0.32	62.2 ± 1.85
Fischer 344 (stimulated)	2	81.7 ± 5.14	79.6 ± 2.10	79.2 ± 1.65	11.5 ± 1.04
	4	65.2 ± 6.68	57.4 ± 2.91	81.4 ± 0.20	28.5 ± 1.47
	8	53.5 ± 1.68	30.8 ± 3.24	82.3 ± 0.85	54.5 ± 2.30
Buffalo (normal)	2	58.8 ± 10.9	55.8 ± 1.76	63.3 ± 1.05	26.9 ± 2.10
	4	50.6 ± 3.75	40.8 ± 1.50	69.4 ± 0.15	53.1 ± 4.37
	8	54.7 ± 7.60	22.7 ± 3.41	71.4 ± 1.95	97.4 ± 4.88
Buffalo (stimulated)	2	91.2 ± 4.80	92.6 ± 10.7	73.4 ± 4.08	21.3 ± 1.87
	4	57.7 ± 11.5	60.4 ± 3.97	77.4 ± 1.55	38.2 ± 4.20
	8	61.1 ± 7.33	28.0 ± 6.20	78.3 ± 1.72	90.8 ± 2.10

[a] See footnotes to Table 5.

[b] Adherent cells obtained from normal peritoneal washing or a peritoneal exudate stimulated by injection of thioglycolate.

rial soluble in acid and able to diffuse from the macrophage into the medium. This, however, can only reflect digestion of some unknown protein and is not a measure of degradation of bacterial cells. Ayoub and Wannamaker (1) studied the fate of ^{32}P- and ^{14}C-labeled group A streptococci following phagocytosis by human leukocytes. After 12 h in culture, approximately 20% of the ^{32}P and 10% of the ^{14}C from labeled streptococcal cells were released in a dialyzable form as a result of phagocytic degradation. Ginsburg and co-workers have compared lysis

of several bacterial species by extracts of leukocytes (8). They also report that extracts of macrophages are less bacteriolytic than polymorphonuclear leukocytes. Recently Gallis et al. (6) reported that native radiolabeled group A streptococci are resistant to degradation by egg lysozyme and human lysosomal enzymes. Only after *N*-acetylation of the cell walls was there any significant degradation by these enzymes. The limitations of these studies are that the isotopes cannot be related to specific bacterial structures, and/or the studies were very short-term. Thus, they provide no direct information on the capacity of identifiable bacterial components to resist degradation and persist for extended periods within tissue or phagocytic cells. We report here that the cell walls of group A streptococci that are preferentially labeled in the peptidoglycan or polysaccharide moieties with either [^{14}C]alanine or [^{14}C]glucose, respectively, are not appreciably degraded by cultured macrophages for at least 8 days after phagocytosis. In fact, in vitro phagocytized group A streptococcal cell walls were demonstrated by immunofluorescent microscopy to persist undiminished in cultured rat macrophages for at least 40 days and in human monocytes for at least 24 days. Group D streptococcal cell walls, however, are eliminated by 8 days in cultured rat macrophage and by 6 days in cultured human monocytes. These in vitro observations confirm and extend in vivo findings on the fate of streptococcal cell walls (10, 11, 14).

After a single intraperitoneal or intravenous injection of group A streptococcal cell wall fragments, rats of several strains, including Fischer 344 rats, develop inflammation, bone erosion, and ankylosis of joints similar to that found in rheumatoid arthritis in humans (Anderle and Cromartie, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, B49, p. 19). However, rats of the Buffalo strain are much less susceptible. Furthermore, whereas group A streptococcal cell wall antigens could be demonstrated in the joints of Fischer 344 rats, no cell wall material could be found in the joints of Buffalo rats (Anderle and Cromartie, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, B49, p. 19). In vitro culture techniques were developed to help determine if this difference in susceptibility reflects a difference between abilities of macrophages from these rats to phagocytize or degrade streptococcal cell walls. The phagocytosis of group A and group D streptococcal cell walls by macrophages obtained from Fischer 344 or Buffalo rats was found to be essentially the same. Similarly, no appreciable difference in the ability to degrade group A streptococcal cell walls by cultured macrophages from these two rat strains was observed. However, Buffalo macrophages were found to degrade group D streptococcal cell walls faster and to a greater extent than Fischer 344 macrophages. This may represent a functional difference in the degradative capacity of macrophages from Buffalo rats not apparent with more resistant group A cell walls. It is not known if macrophages from Buffalo rats contain higher levels of lysozyme, which degrades group D streptococci (7).

These observations do not explain the fact that group A cell walls are not found in the joints of Buffalo rats. The translocation of macrophages that have phagocytized bacterial cell walls may be the critical factor in determining if this material localizes in a target organ or tissue with subsequent development of chronic inflammation. Different species have different sites of localization of these cell walls, which subsequently give rise to chronic inflammation. Whereas intraperitoneal injection of rats gives rise to joint involvement, mice develop rheumatic-like heart lesions (4, 11, 14). Using another approach, we have shown that Fischer 344 macrophages containing phagocytized group A cell walls are cytotoxic for L-cells, whereas Buffalo macrophages and macrophages from either strain containing the degradable group D cell walls are not cytotoxic (19).

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