INFECTION AND IMMUNITY, July 1985, p. 116–121
0019-9567/85/070116-06$02.00/0

Vol. 49, No. 1

Susceptibility of *Propionibacterium acnes* to Killing and Degradation by Human Neutrophils and Monocytes In Vitro

GUY F. WEBSTER,[1]* JAMES J. LEYDEN,[1] ROBERT A. MUSSON,[2] AND STEVEN D. DOUGLAS[2]

Department of Dermatology, University of Pennsylvania,[1] and Department of Pediatrics, Children's Hospital of Philadelphia,[2] Philadelphia, Pennsylvania 19104

Received 14 January 1985/Accepted 25 March 1985

***Propionibacterium acnes*, the target of inflammation in acne, was tested for its sensitivity to the bactericidal and degradative functions of human polymorphonuclear leukocytes (PMN), monocytes, and their fractions. *P. acnes* strains were not killed by PMN under any conditions and were variably killed by monocytes in the presence of serum from acne patients. Control strains of *Staphylococcus aureus* and *Micrococcus lysodeicticus* were susceptible to both PMN and monocyte killing. *P. acnes* strains were also not killed by lysozyme, chymotrypsin, H_2O_2, human serum, PMN granule lysate, and PMN and monocyte cell lysates. The organism was sensitive to the bactericidal activity of myeloperoxidase in acid pH. In addition, *P. acnes* was shown to be relatively resistant to the degradative action of PMN and monocyte lysates, whereas *M. lysodeicticus*, *S. aureus*, and *Staphylococcus epidermidis* were all degraded to various degrees. The moieties that were liberated from *P. acnes* by PMN enzymes were predominantly low in molecular weight (1,000 to 25,000) and were consistent with cell wall fragments.**

Propionibacterium acnes is the major, if not only, inhabitant of the human sebaceous follicle and has been shown to play a central role in the production of inflammatory acne lesions (8). The organism is a potent inflammatory stimulus in that it can activate complement by both classical and alternative pathways (26, 28), produce serum-independent polymorphonuclear leukocyte (PMN) chemotactic factors (16, 25), and trigger externalization of PMN hydrolytic enzymes (27). Furthermore, the development in vivo of inflammatory acne lesions is dependent on the presence of high comedonal *P. acnes* populations, and successful therapy of inflammatory acne requires the suppression of these bacteria (8).

Although the mechanisms by which *P. acnes* can cause the acute aspects of cutaneous inflammation have been investigated, there is no explanation for the unusual persistence of acne lesions, which often evolve into foci of chronic inflammation (8). Since the persistence and severity of a bacterial lesion may be in part determined by the ability of the host to kill and degrade the organism, and thus remove the inflammatory stimulus, we have studied the interaction between *P. acnes* and normal human leukocytes.

MATERIALS AND METHODS

Materials. Human PMN myeloperoxidase (MPO) was partially purified from an acid extract of human leukocytes by column chromatography and was supplied by Norman Schecter. Before use, the MPO was dialyzed against three changes of distilled water for 48 h at 4°C. Carbohydrate was assayed by the phenol-sulfuric acid method. MPO activity was assayed by the guaiacol assay of Paul et al. (14). Lysozyme activity was assayed by the agarose plate method (13). Guaiacol, elastase, bovine pancreatic chymotrypsin, agarose, dried *Micrococcus lysodeicticus* cells, human milk lysozyme, sodium azide, hydrogen peroxide, and trypan blue were obtained from Sigma Chemical Co., St. Louis, Mo.

Serum from persons with severe nodulocystic acne (acne patient serum) was collected, heat inactivated at 56°C for 30 min, and pooled. At the time of collection patients were not taking any prescription medications. The anti-*P. acnes* ATCC 6919 agglutination titer of the serum pool was 1:1,024, and the anti-*P. acnes* ATCC 6919 carbohydrate precipitin titer was 1:128 (G. F. Webster, J. Indrisano, and J. J. Leyden, J. Invest. Dermatol., in press).

The gamma globulin fraction of normal and acne patient serum was precipitated with ammonium sulfate by standard methods (6). Purity was assessed by immunoelectrophoreses developed with antiserum to whole human serum, human immunoglobulins, and human ceruloplasmin (Calbiochem-Behring, La Jolla, Calif.). Before use, the normal serum and acne patient serum gamma globulin preparations were standardized according to protein content by the Folin phenol reaction (12).

Bacteria. *P. acnes* strains ATCC 6919 (American Type Culture Collection) and VPI 3706, 0400, 3318, 2742, 6639, and 6578 (Virginia Polytechnic Institute; obtained from C. S. Cummins) were grown under anaerobic conditions in peptone-yeast-glucose broth with 0.1% Tween 80 as previously described (28). *Micrococcus lysodeicticus* ATCC E4698, *Staphylococcus epidermidis* 6007 (Duhring Laboratories culture collection), and *Staphylococcus aureus* 97 (Duhring Laboratories culture collection), were cultured on tryptic soy agar or broth (Baltimore Biological Co.).

Bacteria were quantified by counting the number of CFU in samples of serial dilutions. The logarithm of the number of CFU was derived, and data are expressed as the (log experimental)/(log control) (%log) to minimize the effect of trivial variations in bacterial viable counts on calculated experimental results. By this method a reduction of bacteria from 10^6 to 10^4 would be a 33 %log reduction.

Preparation of phagocytes. PMN and monocytes were collected from the heparinized peripheral blood of normal human volunteers who had no history of significant inflammatory acne. Cells were retrieved from buffy coat, washed in 0.15 M NaCl, and then purified on a Ficoll-sodium diatrizoate separation medium (Sigma). This procedure resulted in a PMN preparation that was 98% free of contaminating monocytes and lymphocytes and a mononuclear cell

* Corresponding author.

preparation that was 99% free of PMN. Cells were adjusted to a concentration of 5×10^6 in Hanks balanced salts solution with 1% fetal calf serum (GIBCO Laboratories, Grand Island, N.Y.). PMN and monocyte viability was greater than 97% as assessed by trypan blue exclusion. Bactericidal activity was assessed at a PMN/bacteria ratio of 50:1 and a monocyte/bacteria ratio of 10:1. Bacteria and phagocytes were incubated at 37°C on a shaking bath at 60 rpm.

PMN granules were purified by homogenization of PMN in 25% sucrose to 85 to 90% breakage in a Teflon-glass homogenizer followed by differential centrifugation by established methods (23). The granule preparation was then subjected to five cycles of freezing and thawing.

Degradation of bacteria. Degradation was assessed by two independent methods, decrease in optical density and liberation of radiolabeled cell components. Turbidometric studies were performed by comparing the optical density at 540 nm of treated and untreated suspensions of bacteria. Bacteria were uniformly radiolabeled with ^{14}C by growth in peptone-yeast-glucose broth (28) with 0.2% glucose and 1 μCi of [^{14}C]glucose (specific activity, 4 to 5 mCi/mmol; New England Nuclear Corp., Boston, Mass.) per ml (1, 5, 9, 10). *P. acnes* strains were cultured for 72 h, and all other strains were cultured for 24 h at 37°C. After the culture period the labeled bacteria were retrieved by centrifugation at 2,000 × *g*, the supernatant was discarded, and the cell pellet was washed five times at 5000 × *g*. The final wash supernatant had less than 300 cpm of ^{14}C per ml. Washed cells (optical density of 0.5 at 540 nm) were then incubated with various preparations of leukocyte components. After appropriate periods the incubation mixture was centrifuged at 10,000 × *g*, and the supernatant was filtered through a 0.45-μm membrane filter (Millipore Corp., Bedford, Mass.) to remove cells and large cell fragments. The amount of ^{14}C in the supernatants was counted in a Packard Tricarb 300 scintillation counter with Aquafluor 2 scintillation fluid (New England Nuclear). Controls included bacteria incubated with all components of the reaction mixture except leukocyte-derived compounds. The difference in counts between experimental bacteria and controls was taken as a measure of the extent of degradation.

Column chromatography was performed in a 55- by 1-cm Sephadex G-50 column eluted with 0.15 M saline. *P. acnes* digests were centrifuged and filtered through a 0.45-μm membrane before chromatography.

P. acnes strains were radiolabeled by growth in [^{14}C]glucose-containing media. The distribution of ^{14}C in *P. acnes* 6919 was determined by extraction of cells with chloroform-methanol for lipids, followed by 5% trichloracetic acid at 56°C for 18 h and precipitation with acetone for carbohydrate. Extraction with lipid solvents removed 1.5% of the label, extraction and precipitation of cell carbohydrate recovered 48.1% of the label, and 34.8% of the label remained in the insoluble cell residue. The total recovery of ^{14}C was 84.5%.

RESULTS

Phagocyte bactericidal activity. *P. acnes* ATCC 6919 and VPI 0400, *S. aureus* 97, and *M. lysodeicticus* were incubated with human PMN at a ratio of 50 PMN per bacterium for 60 and 120 min at 37°C with and without APS or autologous serum. After incubation, 0.1 ml of 0.5% Triton X-100 was added to lyse PMN, and the number of viable bacteria was determined. The number of viable bacteria is presented as the percentage of the logarithm of CFU in the test and PMN-free incubation mixtures. *P. acnes* strains were not killed by PMN in any incubation mixture. *S. aureus* viability was reduced to 71 %log in 60 min and 56.8 %log in 120 min when active complement was present in the incubation mixture. *M. lysodeicticus* was killed 100% by PMN under all incubation conditions (Fig. 1). Further study with *P. acnes* strains VPI 3318, 2742, 6639, and 6578 revealed that these strains were also resistant to killing by PMN even when fresh serum and acne patient serum were present.

The ability of human monocytes to kill *P. acnes* was assessed at a monocyte/*P. acnes* ratio of 10:1, with a 3-h incubation at 37°C (Fig. 2). Monocytes reduced *P. acnes* viability (to 62 %log for strain 400 and 75 %log for strain ATCC 6919) only when acne patient serum was present. *S. aureus* was reduced to 63 %log when active complement was present, and *M. lysodeicticus* was completely killed by all monocyte incubation mixtures.

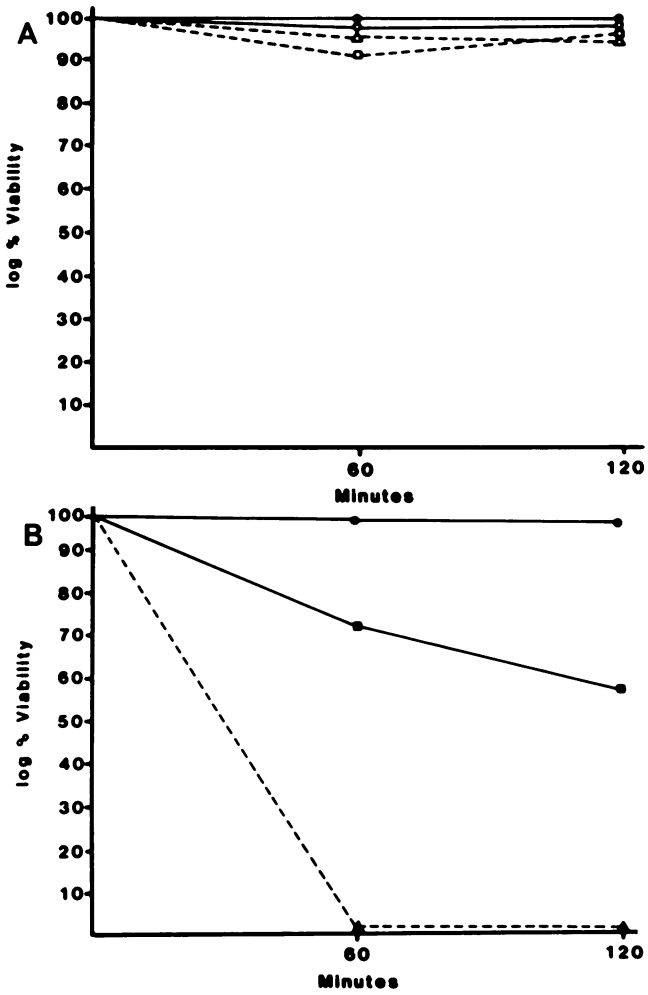


FIG. 1. (A) PMN bactericidal activity against *P. acnes*. Symbols (standard deviations): ●, ATCC 6919 with fresh normal serum (<1.5); ○, ATCC 6919 plus acne patient serum (<3.3); □, VPI 0400 plus fresh normal serum (<5.0); △, VPI 0400 plus acne patient serum (<6.0). The data are the means of a minimum of three repetitions. (B) PMN bactericidal activity against control organisms. Symbols (standard deviations): ●, *S. aureus* plus acne patient serum (<1); ■, *S. aureus* plus fresh normal serum (<3.3); ▲, *M. lysodeicticus* plus fresh or acne patient serum (0). Data are the means of a minimum of three repetitions.

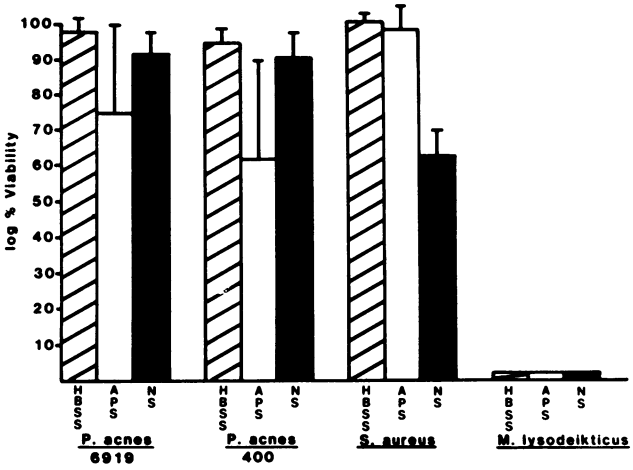


FIG. 2. Monocyte bactericidal activity. Abbreviations: HBSS, Hanks balanced salts solution; APS, acne patient serum; NS, fresh normal serum. Comparative viability after incubation with PMN for *P. acnes* ATCC 6919 plus acne patient serum was 98.9, that for *P. acnes* VPI 0400 plus acne patient serum was 97.0, that for *S. aureus* plus normal serum was 56.8, and that for *M. lysodeicticus* was 0. The data are the means of a minimum of three repetitions.

Enzymatic antibacterial activity. The ability of hydrolytic enzymes to kill *P. acnes* in vitro was also tested. Bacteria were incubated at 37°C for 30 and 60 min with 50% human serum, 50 μg of human milk lysozyme per ml, 50 μg of bovine pancreatic chymotrypsin per ml, 10 mM hydrogen peroxide, and human PMN MPO at pH 5.0, 6.0, or 7.0 in phosphate- or citrate-buffered saline (Table 1). *P. acnes* and *S. aureus* strains were not killed by chymotrypsin, lysozyme, or hydrogen peroxide. *M. lysodeicticus* was 100% killed by lysozyme and 7.2% killed by H_2O_2 but not killed by chymotrypsin. All strains were killed by 2 U of MPO plus 10^{-4} M H_2O_2 at pH 5.0. MPO-mediated killing was further investigated with *P. acnes* strain ATCC 6919. The enzyme system was bactericidal after 30 min at pH 5.0 and 6.0, but not pH 7.0. Killing was rapid and complete within the first 5 min of incubation at pH 5.0, and as little as 0.125 U of MPO was effective in killing *P. acnes* at pH 5.0 and 6.0. Bactericidal activity at pH 5.0 was completely inhibited by the addition of the MPO inhibitor sodium azide (0.015 M) to the incubation mixtures. Killing did not take place if H_2O_2 or Cl^- was eliminated from the incubation mixture. The addition of purified human anti-*P. acnes* immunoglobulin to the MPO-H_2O_2-Cl^- mixtures failed to protect *P. acnes* from killing.

PMN and monocyte lysates were prepared by the addition of 0.5% Triton X-100 to cells or repeated freezing and thawing of cell suspensions. Different lysate preparations were standardized such that each contained 50 μg of lysozyme activity per ml. This resulted in preparations containing approximately 1 U of MPO activity per ml. These preparations readily killed *M. lysodeicticus*, but failed to kill *P. acnes* or *S. aureus* even after the addition of hydrogen peroxide (Table 1). PMN granule lysate also failed to kill *P. acnes* and *S. aureus*, but readily killed *M. lysodeicticus*.

The ability of PMN and monocyte enzymes to inhibit *P. acnes* growth was assessed by incubation of bacteria with PMN and monocyte Triton X-100 lysates in a defined tissue culture medium (CMRL 1066; GIBCO), to avoid interaction of the leukocyte enzymes with the complex constituents of normal bacterial growth media. The numbers of *P. acnes* and *S. aureus* after 24 h of incubation with leukocyte enzymes were compared with preincubation populations and an incubated, enzyme-free control. Leukocyte enzymes had no effect on the proliferation of *P. acnes* or *S. aureus* (data not shown).

Degradation by phagocyte enzymes. Degradation was assessed by reduction in optical density of treated suspensions of bacteria and by release of ^{14}C from radiolabeled bacteria. PMN lysates were prepared by Triton X-100 treatment and were adjusted to contain equal amounts of lysozyme. The results of the turbidometric studies are presented in Fig. 3. *P. acnes* strains ATCC 6919 and VPI 0400, 3318, and 6578 were all resistant to degradation by PMN lysate at pH 5.0 or 6.0 after up to 24 h of incubation. Treatment of *P. acnes* with lysozyme (50 μg/ml) alone resulted in a minimal decrease in turbidity after 24 h (less than 6% in all cases). *S. aureus*, *S. epidermidis*, and *M. lysodeicticus* were all significantly degraded by both PMN lysate and lysozyme at pH 5.0 at 6.0.

Since *P. acnes* strains showed minimal degradation in the turbidimetric assay, the release of radiolabel was chosen as a more sensitive measure of cellular degradation. Labeled *P. acnes* cells were treated at pH 5.0 or 6.0 with PMN or monocyte lysates or human lysozyme. All preparations were adjusted to a final concentration of 50 μg of lysozyme activity per ml. The release of ^{14}C was uniformly less than 10% after 24 h for all strains and conditions tested (Table 2). The release by PMN lysates was greater than that which resulted from treatment with lysozyme or monocyte lysates. The release of label by PMN lysate was no greater at pH 5.0 than at pH 6.0 and was not significantly affected by heat killing the *P. acnes* or treatment of bacteria with MPO and H_2O_2 before incubation with the enzymes. The spontaneous release of label from buffer-treated bacteria was minimal (generally <5%) for 3- to 5-day-old *P. acnes* bacteria, but was severalfold higher for 1-day-old bacteria. This spontaneous release was ablated by preincubating the 1- or 3-day-old bacteria at 56°C for 30 min, indicating that spontaneous release of label was probably due to heat-sensitive bacterial autolysins.

To partially define the size and nature of the moieties that are released from *P. acnes*, strain ATCC 6919 was labeled by

TABLE 1. Enzymatic bactericidal activity

Strain	log % viability (SD)[a]						
	LZM	CT	PMNTx	GranEx	H_2O_2	MPO^+	PMNTx plus H_2O_2
P. acnes ATCC 6919	100.8 (1.3)	99.3 (1.8)	101.3 (2.3)	100.6 (1.7)	100.9 (0.6)	0 (0)	100.7 (2.1)
P. acnes VPI 0400	99.9 (4.9)	98.9 (6.1)	101.7 (0.4)	99.4 (1.4)	98.5 (2.6)	0 (0)	98.9 (0.9)
M. lysodiecticus	0 (0)	102.1 (2.5)	0 (0)	0 (0)	92.8 (8.6)	0 (0)	0 (0)
S. aureus	101.3 (1.5)	100.1 (1.6)	100.4 (1.7)	100.2 (4.5)	100.3 (2.0)	12.2 (9.7)	99.2 (1.9)

[a] Abbreviations: LZM, lysozyme; CT, chymotrypsin; PMNTx, PMN lysed with Triton X-100; GranEx, granule extract; MPO^+, myeloperoxidase plus H_2O_2 and Cl^-. The data are the means of a minimum of three repetitions.

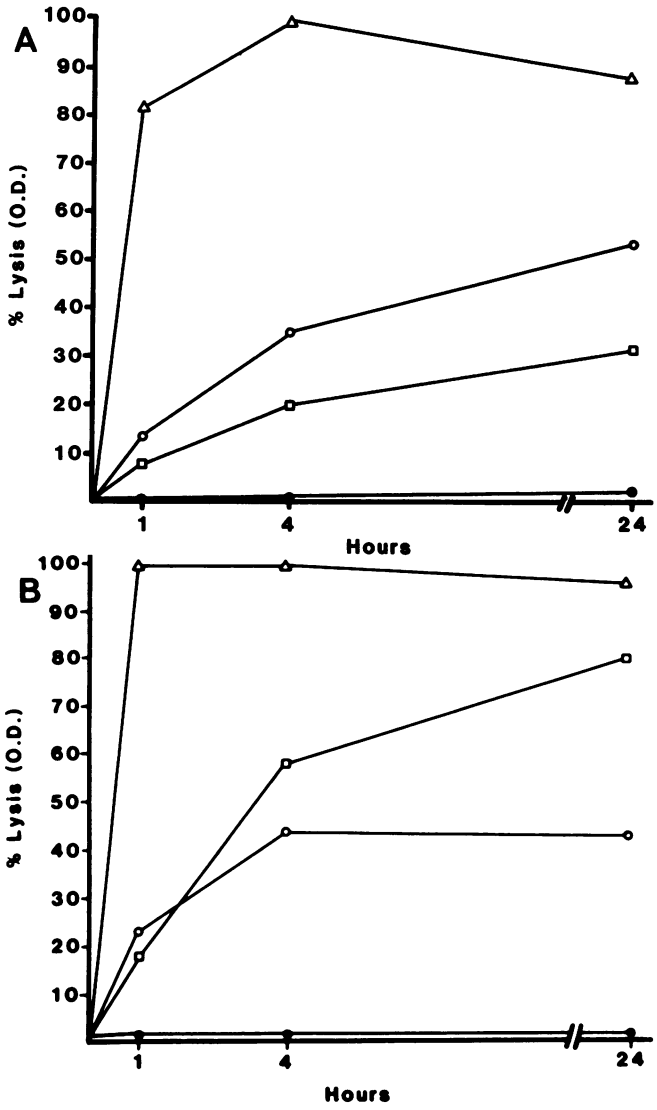


FIG. 3. Turbidimetric bacterial lysis by lysozyme (A) or PMN lysate (B) at pH 6. Symbols: △, *M. lysodeicticus*; □, *S. aureus*; ○, *S. epidermidis*; ●, *P. acnes*.

growth in [^{14}C]glucose and [^{3}H]isoleucine and then digested with PMN lysate at 100 μg of lysozyme per ml. To assure that quenching of ^{14}C could not produce a spurious elevation of ^{3}H counts, the ^{3}H was used at a 10-fold greater activity than the ^{14}C. After incubation for 24 h, the PMN lysate released a small amount of label from *P. acnes* in the void volume and a larger amount of low-molecular-weight material (5,000 to 15,000). Both isotopes had the same elution pattern (Fig. 4A). *P. acnes* incubated in buffer alone produced only the void volume peak (Fig. 4B). Chemical quantification of carbohydrate failed to reveal any unlabeled carbohydrate peaks. Digestion of [^{14}C]glucose-labeled *P. acnes* ATCC 6919 with monocyte lysate produced a similar elution profile (Fig. 4C), as did PMN lysate digestion of *P. acnes* VPI 0400.

DISCUSSION

The function of PMN and monocytes/macrophages in inflammation is twofold: to kill invading microorganisms and to blunt the inflammatory response by removal of the inflammatory stimulus. Persistence of the inflammatory stimulus results in long-standing inflammation or in the development of a granuloma. If the cause of the inflammation is bacterial, then failure to either kill or degrade the organism may result in a persistent lesion (22). This is apparently the case with certain microorganisms, for example, *Mycobacterium tuberculosis*. Although mycobacteria are susceptible to oxidative killing in vitro, they persist viable and undegraded in cells or in tissue and incite an ongoing immune granuloma (11). Similarly, group A streptococci are killed, but resist degradation and incite a more persistent inflammation than more digestable streptococci (21).

We have shown that *P. acnes* is resistant to killing by human PMN and monocytes. Under conditions that result in rapid phagocytosis (27), PMN were completely unable to kill six strains of *P. acnes*. These same conditions resulted in the efficient killing of *S. aureus* and *M. lysodeicticus*. Similarly, monocytes were only able to kill a small proportion of the *P. acnes* strains, although non-*P. acnes* strains were readily killed.

PMN and monocyte lysates, H_2O_2, lysozyme, and chymotrypsin did not kill *P. acnes*; however, purified MPO-H_2O_2-Cl^- did kill *P. acnes* and control organisms. Bactericidal activity was rapid, required H_2O_2, and was inhibited by the addition of azide, an inhibitor of MPO (7, 17). MPO-mediated killing was only active at acidic pH, a condition which enhances the affinity of Cl^- for MPO and its ability to chlorinate in vitro (24). The reason for the inability of PMN lysates with demonstrated MPO peroxidase activity to kill *P. acnes* when H_2O_2 was added is not apparent, but may indicate the presence of inhibitors of chlorination in the lysate or chlorination of PMN proteins in the lysate rather than *P. acnes*. The addition of protein (in the form of purified human anti-*P. acnes* immunoglobulin) to purified MPO-H_2O_2-Cl^- failed to protect *P. acnes* from killing by MPO.

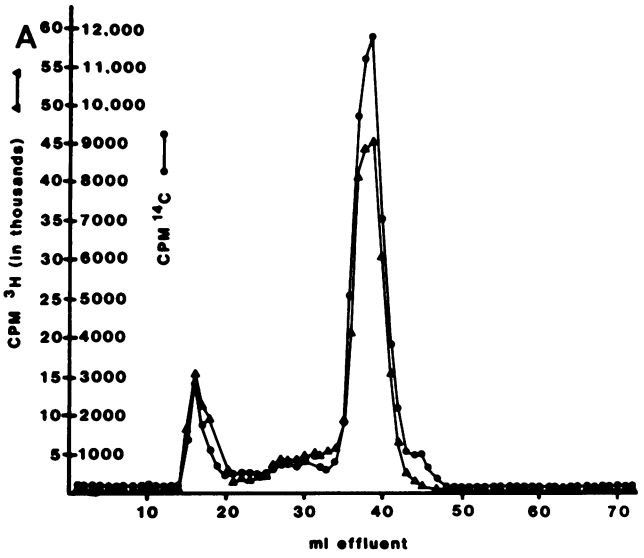
P. acnes was also very resistant to degradation by leukocyte enzymes. Under conditions that produced 40 to 100% lysis of *S. aureus*, *S. epidermidis*, or *M. lysodeicticus*, *P. acnes* was only minimally lysed. The release of ^{14}C-labeled *P. acnes* compounds was also minimal and could not be increased by previous heat killing or treatment of the bacteria with MPO-H_2O_2-Cl^-.

Column chromatography of the structures released from *P. acnes* by PMN enzymes revealed two peaks of radiolabel. The lower-molecular-weight peak was found only in enzymatically treated *P. acnes*, suggesting that the structures

TABLE 2. Degradation of *P. acnes* by PMN and monocyte lysates

P. acnes strain	pH	% of total ^{14}C release[a]			
		PMN-Tx	FT-PMN	MN-Tx	Lysozyme
ATCC 6919	5.0	7.8	5.3	5.5	4.0
ATCC 6919	6.0	9.4	0.9	6.1	2.5
VPI 0400	5.0	4.7	1.5	0.9	1.6
VPI 0400	6.0	4.0	6.7	0.2	1.6
VPI 3318	5.0	6.0		1.8	2.0
VPI 3318	6.0	8.3		1.1	0.4
VPI 6578	5.0	5.6		4.1	2.2
VPI 6578	6.0	4.6		2.8	0.8

[a] Abbreviations: PMN-Tx, PMN lysed with Triton X-100; FT-PMN, PMN lysed by freeze-thawing; MN-Tx, mononuclear cells lysed by Triton X-100. All lysates were adjusted to 50 μg of lysozyme per ml and were incubated with *P. acnes* for 24 h. The data presented are the means of at least three experiments. Standard deviations are less than or equal to 3.0.



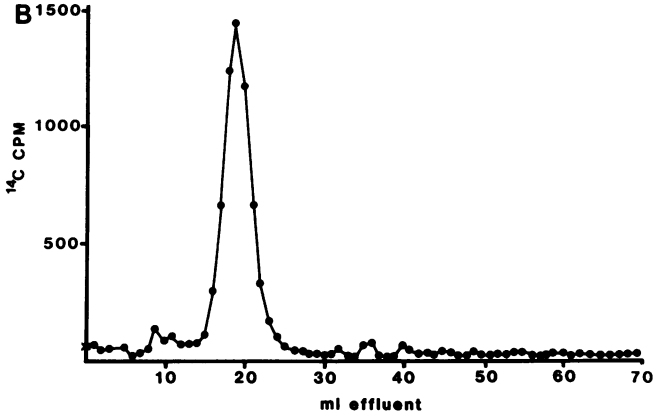
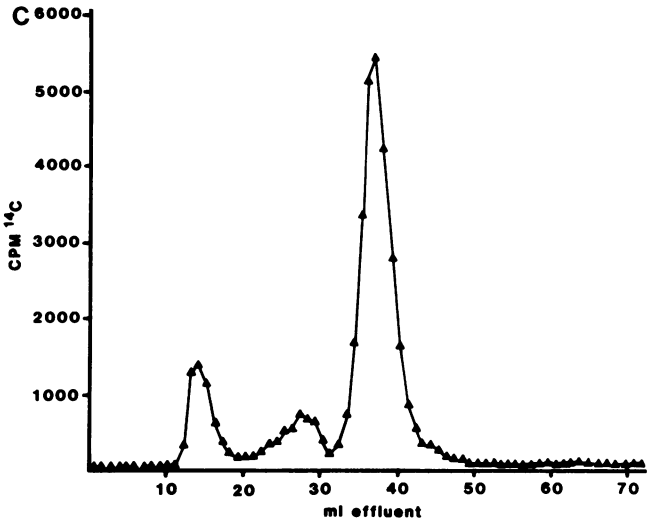


FIG. 4. (A) Sephadex G-50 filtration of [^{14}C]glucose- and [^{3}H]isoleucine-labeled *P. acnes* cells digested with PMN lysate at pH 6.0. Molecular weight markers: blue dextran (void volume), 16 ml of effluent; ovalbumin (45,000), 22 ml of effluent; tetracycline (490), 49 ml of effluent. (B) Sephadex G-50 filtration of ^{14}C spontaneously released by *P. acnes*. (C) Sephadex G-50 filtration of [^{14}C]glucose-labeled *P. acnes* cells digested with monocyte lysate at pH 6.0.



were not released solely by the action of bacterial autolysins. All of the fragments cleaved from *P. acnes* ATCC 6919 were labeled by both radioactive glucose and amino acid, which suggests that they represent cell wall structures containing amino sugars such as *N*-acetylglucosamine. Monocyte lysate produced a similar digest of strain ATCC 6919, as did PMN lysate treatment of *P. acnes* VPI 0400. There was no evidence that leukocyte enzymes degraded *P. acnes* components into individual monomers or disaccharides as has been reported for gonococcal peptidoglycan (18, 19).

The basis for the resistance of *P. acnes* to degradation is not known, but may relate to its secondary cell wall structure. Studies of gonococci whose peptidoglycan resists lysozyme have revealed that they differ from sensitive strains in that there is extensive O-acetylation of the resistant peptidoglycan (18, 19).

Although the degradability of *P. acnes* in vivo has not been studied, there is evidence that suggests that *P. acnes* may persist in tissue. While studying the lymphoreticular stimulator *Corynebacterium parvum*, Dimitrov and colleagues (3) injected mice with *C. parvum* (a synonym for *P. acnes* [3]), whose protein was labeled with ^{125}I, and monitored its organ distribution. They found persistent radioactivity in spleen, liver, and thymus greater than 1 week after injection. Similarly, Sadler and co-workers (20) injected mice with [^{3}H]thymidine-labeled *C. parvum* cells and found that label could be recovered from the injection site and the liver greater than 2 weeks after administration. Finally, an electron microscopic study by Pringle et al. (15) showed that, in vitro, some strains of *P. acnes* appear undegraded 24 h after phagocytosis by murine macrophages. The relevance of these murine studies to human acne is limited, since no other bacteria was compared with *P. acnes*. Moreover, all studies used formalin or heat treatment to kill the bacteria (which may alter degradability, e.g., through inactivation of autolysins), and none radiolabeled the *P. acnes* cell wall (which would presumably be least degradable).

The ability of *P. acnes* to persist undegraded in tissue may in part be the cause of the longevity of inflammatory acne lesions. It has been observed that after the start of antibiotic therapy very few new acne lesions appear, but the established lesions still take some time to resolve (8). This resistance to therapy may actually reflect the delay in degradation of lesional *P. acnes*.

ACKNOWLEDGMENTS

This work was supported in part by Public Health Service research grants AM27213 from the National Institute of Arthritis, Metabolism and Digestive Diseases and 5-T32-HD-07217-03 from the National Institutes of Health.

LITERATURE CITED

1. **Cohn, Z. A.** 1963. The fate of bacteria within phagocytic cells. I. The degradation of isotopically-labelled bacteria by polymorphonuclear leukocytes and macrophages. J. Exp. Med. **117**:27–42.
2. **Cummins, C. S., and J. L. Johnson.** 1974. *Corynebacterium parvum*: a synonym for *Propionibacterium acnes*? J. Gen. Microbiol. **80**:433–442.
3. **Dimitrov, N. V., C. S. Greenberg, and T. Denny.** 1977. Organ distribution of *Corynebacterium parvum* labeled with ^{125}I. J. Natl. Cancer Inst. **58**:287–294.
4. **Dubois, M., R. A. Gilles, J. K. Hamilton, P. A. Rebers, and F.**

Smith. 1956. Colorimetric method for the determination of sugars and related substances. Anal. Chem. **23**:350–359.
5. **Gallis, H. A., S. E. Miller, and R. W. Wheat.** 1976. Degradation of ^{14}C-labeled streptococcal cell walls by egg white lysozyme and lysosomal enzymes. Infect. Immun. **13**:1459–1466.
6. **Garvey, J. S., N. E. Cremer, and D. H. Sussdorf.** 1977. Methods in immunology, 3rd ed., p. 218–219. Addison-Wesley Co., New York.
7. **Klebanoff, S. J.** 1968. Myeloperoxidase-halide-hydrogen peroxide antibacterial system. J. Bacteriol. **95**:2131–2138.
8. **Kligman, A. M.** 1974. An overview of acne. J. Invest. Dermatol. **62**:268–287.
9. **Lahav, M., N. Ne'eman, E. Adler, and I. Ginsburg.** 1974. The effect of leukocyte hydrolases on bacteria. I. Degradation of C-labelled *Streptococcus* and *Staphylococcus* by leukocyte lysates in vitro. J. Infect. Dis. **129**:528–537.
10. **Lahav, M., N. Ne'eman, J. James, and I. Ginsburg.** 1975. The effect of leukocyte hydrolases on bacteria. III. Bacteriolysis induced by extracts of different leukocyte populations and the inhibition of lysis by macromolecular substances. J. Infect. Dis. **131**:149–157.
11. **Lowrie, D. B.** 1983. Mononuclear phagocyte-mycobacterium interaction, p. 235–278. *In* C. Ratledge and J. Stanford (ed.), Biology of the mycobacteria, vol. 2. Academic Press, Inc., New York.
12. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
13. **Osserman, E. F., and D. P. Lawlor.** 1966. Serum and urinary lysozyme (muramidase) in monocytic and monomyelocytic leukemia. J. Exp. Med. **125**:921–952.
14. **Paul, B. B., R. J. Selvaraj, and A. J. Sbarra.** 1978. A sensitive assay method for peroxidases from various sources. RES J. Reticuloendothel. Soc. **23**:407–410.
15. **Pringle, A. T., C. S. Cummins, B. F. Bishop, and V. S. Viers.** 1982. Fate of vaccines of *Propionibacterium acnes* after phagocytosis by murine macrophages. Infect. Immun. **38**:371–374.
16. **Puhvel, S. M., and M. Sakamoto.** 1978. The chemoattractant properties of comedonal contents. J. Invest. Dermatol. **71**:324–329.
17. **Rosen, H., and S. J. Klebanoff.** 1979. Bactericidal activity of a superoxide anion-generating system. J. Exp. Med. **149**:27–39.
18. **Rosenthal, R. S., J. K. Blundell, and H. R. Perkins.** 1982. Strain-rekated differences in lysozyme sensitivity and extent of O-acetylation of gonococcal peptidoglycan. Infect. Immun. **37**:826–829.
19. **Rosenthal, R. S., W. J. Folkening, D. R. Miler, and S. C. Swim.** 1983. Resistance of O-acetylated peptidoglycan to human peptidoglycan-degrading enzymes. Infect. Immun. **40**:903–911.
20. **Sadler, T. E., W. A. Cramp, and J. E. Castro.** 1977. Radiolabelling of *Corynebacterium parvum* and its distribution in mice. Br. J. Cancer **35**:357–368.
21. **Smialowicz, R., and J. H. Schwab.** 1977. Processing of streptococcal cell walls by rat macrophages and human monocytes invitro. Infect. Immun. **17**:591–598.
22. **Spector, W. G., N. Reichhold, and G. B. Ryan.** 1970. Degradation of granuloma-inducing microorganisms by macrophages. J. Pathol. **101**:339–354.
23. **Spitznagel, J. K., F. G. Dalldorf, M. S. Leffell, J. D. Folds, I. R. H. Welsh, M. H. Cooney, and L. E. Martin.** 1974. Character of specific and azurophil granules purified from human polymorphonuclear leukocytes. Lab. Invest. **30**:774–785.
24. **Stelmaszynska, T., and J. M. Zgliczynski.** 1974. Myeloperoxidase of human neutrophilic granulocytes as chlorinating enzyme. Eur. J. Biochem. **45**:305–312.
25. **Webster, G. F., and J. J. Leyden.** 1980. Production of serum-independent polymorphonuclear leukocyte chemotactic factors by *Propionibacterium acnes*. Inflammation **4**:261–269.
26. **Webster, G. F., J. J. Leyden, M. E. Norman, and U. R. Nilsson.** 1978. Complement activation in acne vulgaris, in vitro studies with *Propionibacterium acnes* and *Propionibacterium granulosum*. Infect. Immun. **22**:523–529.
27. **Webster, G. F., J. J. Leyden, C.-C. Tsai, P. Baehni, and W. P. McArthur.** 1980. Polymorphonuclear leukocyte lysosomal release in response to *Propionibacterium acnes* in vitro and its enhancement by sera from patients with inflammatory acne. J. Invest. Dermatol. **74**:398–401.
28. **Webster, G. F., U. R. Nilsson, and W. P. McArthur.** 1981. Activation of the alternative pathway of complement in human serum by *Propionibacterium acnes* (*Corynebacterium parvum*) cell fractions. Inflammation **5**:165–176.