Complement Receptors on Normal Human Lymphocytes Containing Parallel Tubular Arrays

Claire M. Payne, PhD, and Raymond B. Nagle, MD, PhD

Membrane complement receptors have been identified on a subpopulation of normal lymphocytes containing cytoplasmic inclusions called parallel tubular arrays (PTA) using two different rosetting techniques. The first technique utilizes as indicator cells erythrocytes that were coated with complement by the classic pathway of complement activation (EAC rosettes). The second technique utilizes as indicator cells *Salmonella typhi*, which were coated with complement by the alternate pathway of complement activation (FBC rosettes). In the latter technique, lipopolysaccharide material in the bacterial cell wall directly activates complement without the use of a sensitizing antibody. This eliminates binding of marker particles by lymphocytes having Fc receptors. The presence of PTA lymphocytes at the center of EAC rosettes and FBC rosettes was demonstrated by electron microscopy, indicating that the PTA lymphocyte has a complement receptor. Examination of FBC rosettes revealed that the adherent complement-coated bacteria were usually partially surrounded by pseudopodal extensions of the PTA lymphocyte. In addition, some PTA lymphocytes phagocytized the complement-coated bacteria but not the complement-inactivated bacteria. These phagocytic cells were placed in the lymphocytic series instead of the monocytic series by virtue of complete lack of endogenous peroxidase activity. (Am J Pathol 1980, 99:645-666)

LYMPHOCYTES from the normal human peripheral circulation consist of a heterogeneous population of cells having diverse functions.[1] Those lymphocytes containing parallel tubular arrays (PTA lymphocytes) represent one distinct subpopulation having the common property of steroid resistance.[2] This subpopulation of lymphocytes is currently defined only by its ultrastructural characteristics, since a specific surface marker has not yet been ascribed to it.

Previous ultrastructural studies we have made on lymphocytes from patients with severe combined immunodeficiency disease have indicated that parallel tubular arrays were found in both E and EAC rosette-forming cells.[3] McKenna et al,[4] in a study of infectious mononucleosis, described the ultrastructural appearance of a PTA lymphocyte in an E rosette. The authors did not examine EAC rosettes ultrastructurally because they felt that the EAC rosetting technique measures both complement and Fc receptors. Nevertheless, they conclude that the PTA lymphocyte is most probably a T lymphocyte.

From the Department of Pathology, University of Arizona Health Sciences Center, Tucson, Arizona.

Accepted for publication January 22, 1980.

Address reprint requests to Dr. Claire M. Payne, Department of Pathology, University of Arizona Health Sciences Center, Tucson, AZ 85724.

0002-9440/80/0609-0645$01.00

In this study we have identified membrane complement receptors on PTA lymphocytes derived from healthy individuals, using two different rosetting techniques. The first technique utilizes as indicator cells erythrocytes which were coated with complement by the classic pathway of complement activation (EAC rosettes). The second technique utilizes as indicator cells *Salmonella typhi*, which were coated with complement by the alternate pathway of complement activation (FBC rosettes).[5,6] The lipopolysaccharide on the cell wall of the gram-negative bacterium directly activates complement by the alternate pathway, thereby making unnecessary the use of specific antibody in preparing the cells. In addition, the phagocytic ability of complement-receptor–bearing lymphocytes is shown, and the significance of this rare observation is discussed. As a result of this study, we feel that the PTA lymphocyte should not simply be considered a subset of T lymphocytes.

Materials and Methods

Mononuclear Fraction

Venous blood obtained from healthy adults was drawn into sodium–heparin tubes. To 10 ml whole blood was added 20 ml Hanks' solution (2.3 ml 7.5% $NaHCO_3$ + 50 ml 10× strength Hanks' balanced salt solution + 450 ml distilled H_2O). The diluted blood was placed on a Ficoll-Hypaque density gradient and centrifuged for 20 minutes at 3000 rpm in the Sorvall GLC-swinging bucket clinical centrifuge. The mononuclear fraction was removed from the interface and washed three times with Hanks' solution. The cells were suspended in Hanks' solution at a concentration of 4–5 × 10^6 cells/ml.

Preparation of Complement-Coated Erythrocytes (EAC)

Sheep red blood cells (SRBCs) obtained from Micro Tech Diagnostics, Tucson, Arizona, were washed with sterile saline and resuspended in HBSS to obtain a 10% concentration.[3] The cells were incubated with sheep cell hemolysin (Hyland) to obtain an EA complex. The EA complex was then incubated with fresh human AB, Rh(−) serum to obtain an EAC preparation. The EAC preparation was washed three times in HBSS and adjusted to a 1% concentration.

Preparation of EAC Rosettes

EAC rosetting assays were based on the method of Stjernswärd et al.[7] We incubated 0.25 ml of 1% EAC with 0.25 ml of mononuclear cell suspension for 30 minutes at 37 C. The percentage of rosette-forming cells was determined under a phase microscope after examining 100 cells.

Preparation of Fluoresceinated Bacteria (FB)

Salmonella typhi isolated from a clinical case in our microbiology laboratory and confirmed by the Arizona State Laboratory was provided by Dr. Kenneth Ryan. These organisms were grown in 250 ml flasks of Trypticase soy broth at 37 C overnight. They were killed by autoclaving for 20 minutes, pelleted, and washed three times with phosphate-buffered saline (PBS). The slurry was adjusted turbidimetrically so that a 1 : 10 dilution had an optical density of 2.0 at 540 nm. One volume of the bacterial slurry was diluted with 5

volumes of 0.5 M carbonate-bicarbonate buffer, pH 9.5 (1 volume 0.5 M $NaCO_3$ to 3 volumes 0.5 M $NaHCO_3$). To this alkaline bacterial suspension was added 2 volumes of a 0.03% solution of fluorescein isothiocyanate (FITC), isomer I, in the same buffer. This mixture was incubated for 30 minutes at 37 C and washed three times in 0.15 M veronal-buffered saline solution, pH 7.2, with 0.15 mM $CaCl_2$, 1.0 mM $MgCl_2$, and 0.1% gelatin ($GVBS^{++}$).[8] The fluorescein-labeled bacteria (FB) were divided into 1-ml aliquots and frozen at −70 C for later use.

Preparation of Fluoresceinated Complement-Coated Bacteria (FBC)

One volume of the fluorescein-labeled bacteria (FB) was mixed with an equal volume of fresh normal human serum as a source of complement and incubated at 37 C for 15 minutes. The serum was first tested and found negative for anti-Salmonella agglutinins by the Group D somatic 9,12 and flagellar (d) antigen tests (Lederle Diagnostics). As a control, the FB were incubated with serum depleted of complement by heating at 56 C for 30 minutes. The bacteria were then washed three times with 0.15 M veronal-buffered saline, pH 7.2 ($GVBS^{++}$), and resuspended in 12 times the starting volume.

Preparation of FBC and FB Rosettes

Human peripheral blood mononuclear cells were prepared as outlined above. One volume of mononuclear ($4–5 \times 10^6$ cells/ml) was added to one volume of FBC or FB (4.2×10^8 bacteria/ml) and incubated at 37 C for 30 minutes. Following incubation the mixture was placed on ice. A drop was placed under a coverslip and examined with an ultraviolet microscope with and without a darkfield condenser. The percentage of mononuclear cells having 3 or more FBC or FB on their surface was determined after examining 100 cells. We compared the percentage of FBC rosettes with the percentage of FB rosettes to determine the effectiveness of the complement-coating procedure. The remaining cells were fixed and processed for electron microscopy as described below.

Preparation of Rosettes for Electron Microscopy

When the period of incubation for the EAC, FBC, and FB rosettes was completed, an equal volume of 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) was added to the cell suspension. After 1 hour of fixation at room temperature, the suspension was centrifuged at 1000 rpm in a Sorvall GLC-1 swinging bucket clinical centrifuge for 5 minutes and resuspended in human plasma. The suspension was then transferred to a round-bottomed plastic tube (#2059 tube; Falcon, Oxnard, Calif) and centrifuged at 1000 rpm in the swinging bucket clinical centrifuge for 5 minutes.[9] The supernatant was removed and replaced with cold 3% glutaraldehyde in 0.1 M phosphate buffer. The pellet was fixed for one hour at 4 C, rinsed in 0.1 M phosphate buffer, and postfixed for 1½ hours in 1% osmium tetroxide in 0.1 M phosphate buffer. The pellet was dehydrated through a graded series of alcohol and embedded in Spurr's epoxy.[10]

One-micron sections were stained with toluidine blue and examined with the light microscope. Selected blocks were thin-sectioned by the use of a Sorvall MT2-B ultramicrotome. The ultrathin sections, mounted on uncoated 200-mesh copper grids, were stained with uranyl acetate and lead citrate and lightly carbon-coated before examination with a Hitachi HU-12 electron microscope.

Peroxidase Reaction

The leukocyte fraction was obtained from normal human heparinized venous blood after hydroxyethyl starch sedimentation. The cells were washed twice in Hanks' balanced salt solution and fixed for 1 hour at 4 C in tannic acid aldehyde fixative according to the method of Breton-Gorius et al.[11] The fixative was replaced with 0.1 M phosphate buffer

(pH 7.2) and the cells stored for 24 hours at 4 C before incubation in the peroxidase reaction medium. This medium consisted of 20 mg 3,3'-diaminobenzidine-tetrahydrochloride (Electron Microscopy Sciences, Fort Washington, Pa) in 10 ml 0.05 M tris buffer, pH 7.6, containing 0.01% H_2O_2. The reaction mixture was centrifuged at 3000 rpm for 10 minutes, the pellet discarded, and the supernatant readjusted to pH 7.6 with 1 N NaOH before use. The cells were incubated in the reaction medium for 1 hour at room temperature in the dark and rinsed in two changes of tris buffer. Control cells were incubated in the reaction medium without H_2O_2. The cells were postfixed in 1% osmium tetroxide for 1 hour, dehydrated in a graded series of alcohols, and embedded in Spurr's epoxy. Cells were examined unstained under the electron microscope.

Results

Light Microscopy of Rosetted Preparations

The percentage of EAC rosettes prepared from 5 healthy adults (3 women, 2 men) ranged from 20% to 35%. The percentage of FBC rosettes prepared from 2 healthy adults (1 woman, 1 man) ranged from 15% to 17%. The percentage of FB control rosettes was consistently 6%.

Electron Microscopy of PTA Lymphocyte EAC Rosettes

Electron microscopy was performed on EAC rosettes obtained from 2 healthy adults (1 man, 1 woman). At least 50 rosettes from each were examined under the electron microscope. There was extreme variability in the kinds of attachments observed between erythrocytes and the central rosette-forming cells. Only PTA lymphocyte EAC rosettes are described, since a non-PTA lymphocyte cannot be identified on the basis of only one thin section through its cytoplasm. A PTA lymphocyte was identified by the presence in its cytoplasm of a specific microtubule-like inclusion called the parallel tubular array (Figure 7). A close examination of 30 random photographs of PTA lymphocyte EAC rosettes obtained from both specimens revealed that most erythrocyte profiles were closely apposed to the PTA lymphocyte surface (Figures 1 and 3). Some of these rosette profiles showed smooth, broad areas of attachment, which extended over several microns of membrane surface (Figure 1), whereas others were highly irregular (Figure 3). In the latter, numerous surface microvilli appeared to deform the erythrocyte membrane, giving it a scalloped surface appearance. These differences are most probably a result of the plane of section, since serial sections of the same rosette showed different modes of attachment of the lymphocyte to the same erythrocyte (Figure 3). In striking contrast to the rosettes shown in Figures 1 and 3 are rare rosettes in which most of the attachment sites are out of the plane of section, and the erythrocyte profiles appeared therefore to be further removed from the lymphocyte surface (Figure 2). One of the nine surrounding erythrocytes, however, was intimately attached to the lymphocyte surface by an irregu-

lar, broad zone of contact that extended over several microns of membrane surface. The erythrocyte surface also appeared deformed by the lymphocyte's surface microvilli. No erythrocytes were observed to be either partially ingested or totally phagocytized by any lymphocyte in the preparations.

Electron Microscopy of PTA Lymphocyte FBC Rosettes

Electron microscopy was performed on FBC rosettes obtained from two healthy adults (1 man, 1 woman). At least 50 rosettes from each were examined under the electron microscope. In all PTA lymphocyte FBC rosettes examined the bacteria were located in depressions of the lymphocyte surface (Figure 4). No bacteria were attached by single points of contact to the tips of individual microvilli which projected from the lymphocyte surface. The adherent bacteria were usually partially surrounded by pseudopodal extensions of the PTA lymphocyte. The term "pseudopod" is a more appropriate term than "microvilli," since some PTA lymphocytes appeared to be in the process of phagocytizing the complement-coated bacteria (Figure 6).

After examining 100 lymphocyte cellular profiles from each of both controls, only 4% of the lymphocyte cross-sections (including PTA lymphocytes, Figures 6 and 7) were noted actually to contain phagocytized bacteria. Only those cellular cross-sections that included a full nuclear profile were scored. Lymphocytes were identified at the ultrastructural level by their abundant heterochromatin, lack of dumbbell-shaped granules, and lack of large pinocytotic vesicles, the latter two features being typical of monocytes (Figure 9).

Electron Microscopy of PTA Lymphocyte FB Rosettes

Only 6% of the lymphocytes were found by light microscopy to form rosettes with bacteria that were incubated with complement-inactivated serum. Although 3 or more bacteria appeared to adhere to the lymphocyte surface by light microscopy, no lymphocyte could be found having more than 2 "adherent" bacteria after examining ultrathin sections by electron microscopy. These "adherent" bacteria were not surrounded by pseudopodal extensions. Figure 5 illustrates a rare profile in which one bacterium appears to be touching a microvillous projection of the lymphocyte surface. In addition, no lymphocyte was seen in the process of phagocytizing any bacterium. One percent of the lymphocytes (as determined after examining 200 cells from two different controls) had a bacterium included in a thin section of the cell cytoplasm. These "included"

bacteria were never near or touching the cytoplasmic borders of the lymphocyte but were always surrounded by a large space.

Peroxidase Activity

In order to eliminate the possibility that the phagocytic PTA lymphocytes were monocytic variants, endogenous peroxidase activity observable at the ultrastructural level was determined in two normal individuals (1 man, 1 woman). Peroxidase reaction product could not be found anywhere in the cellular profiles containing parallel tubular arrays (Figure 8). Specifically, no peroxidase-positive granules could be identified within the cytoplasm of these cells. The parallel tubular arrays themselves also displayed no peroxidase activity (Figure 8, inset). In contrast, cells of the monocytic series, with which PTA lymphocytes can be confused, could be readily identified by their numerous peroxidase-positive granules, some of which have a typical dumbbell-shaped appearance (Figure 9). Other cells in the preparation that contained peroxidase activity included polymorphonuclear leukocytes, eosinophils, and platelets. All cells showed nonspecific surface peroxidase activity, presumably due to absorbed hemoglobin from the lysis of some erythrocytes during the preparation.

Discussion

The demonstration of complement receptors on the cell surface is often hindered because specific antibody is usually used to attach complement to the marker cell. Therefore, some or all of the binding could result from the presence of Fc receptors rather than complement receptors.[4] To circumvent this problem, we have prepared complement-coated *Salmonella typhi* organisms in addition to complement-coated erythrocytes to use as our marker particles. The lipopolysaccharide on the cell wall of gram-negative bacteria activates complement by the alternate pathway, and C3b is deposited on the bacterial surface without the use of antibody.[6,12] Gelfand et al [6] could not demonstrate significant amounts of bound IgG on FBC, using radiolabeled antiserum to human immunoglobulin. We have demonstrated the presence of PTA lymphocytes at the center of these FBC rosettes, indicating that the PTA lymphocyte has a true complement receptor. It is most probable, therefore, that the PTA lymphocyte is not simply a subset of T lymphocytes, as suggested by McKenna et al,[4] since T lymphocytes from the peripheral circulation do not have complement receptors. It is possible that the PTA lymphocytes may belong to a third population of lymphocytes (non-T, non-B, or "killer" cells) which have been reported to form spontaneous rosettes with sheep red blood cells.[1] McKenna et al [4] used the sheep erythrocyte rosette as their sole surface

marker test to conclude that the PTA lymphocyte is probably a subset of T lymphocytes. Ross [1] points out that although the sheep erythrocyte rosette test is used extensively to determine T cell proportions, most investigators find that a parallel test for B and K cells, such as a C receptor assay, is a necessary control. An extensive surface marker analysis at the ultrastructural level is therefore necessary before any conclusion as to the nature of the PTA lymphocyte is reached.

Ultrastructural examination of PTA lymphocyte FBC rosettes revealed that the bacteria 1) were located in depressions of the lymphocyte surface, 2) were usually partially surrounded by pseudopodal extensions of the PTA lymphocyte, and 3) were occasionally phagocytized. No such interactions were observed, however, between complement-inactivated bacteria and the lymphocyte surface, even though some binding was observed at the light-microscopic level. This indicates that the complement receptor in some manner activates the flow of cytoplasm sufficient to cause phagocytosis of the complement-coated bacteria. The 6% binding observed by light microscopy when using control bacteria without complement or their surfaces is probably due to some "stickiness" between bacterial cell wall components and the glycocalyx of the lymphocyte surface. It is interesting that no complement-coated erythrocyte was phagocytized by any lymphocyte in the preparations examined. There may be a sufficient difference in the pattern or density of complement deposition on the two surfaces which causes one cell to be phagocytized rather than the other. On the other hand, steric factors due to size differences between the two types of marker cells may be operable. Very little is known about the phagocytic ability of lymphocytes. Trepel et al [13] showed that rat lymphocytes could ingest carbon particles *in vivo*. Elson et al [14] reported no evidence of phagocytic activity by any central rosette-forming cell using anti-D, Rh(+) erythrocytes. Reyes et al [15] described some lymphocytes from patients with rheumatoid arthritis that appeared to be engulfing fragments of IgG-coated, O, Rh(−) erythrocytes. They referred to these lymphocytes as "atypical" or "monocytoid." Many of the lymphocytes at the center of these specific "EA" rosettes contained parallel tubular arrays. Although these rosettes were termed "rheumatoid rosettes" by the authors, phagocytosis or endocytosis of cell fragments may be a property of normal PTA lymphocytes and bear no specific relationship to rheumatoid arthritis.

Although cells containing parallel tubular arrays appear to be phagocytic, they are not members of the monocytic or granulocytic series, as evidenced by the complete lack of peroxidase activity under the experimental conditions used in this study. Some endogenous peroxidases have

been shown to be inhibited by the glutaraldehyde concentrations commonly used to fix cells for ultrastructural examination.[11,16] However, tannic acid aldehyde fixatives have been employed that adequately fix cells for ultrastructural examination and at the same time preserve the enzymatic activity of these sensitive peroxidases.[11,16] In the present case, even though a tannic acid aldehyde fixative was employed, no peroxidase activity could be detected in the lymphocytes containing parallel tubular arrays. In the same preparation, peroxidase reaction product could be found in the dense tubular system of platelets. This served as a good internal control, since platelet peroxidase activity can be easily inhibited by fixatives. Since the PTA lymphocyte has been shown to represent a distinct subpopulation of lymphocytes,[2] the presence of distinct Fc receptors and complement receptors may bear some relationship to its particular function in the immune system.

The ultrastructure of PTA lymphocyte EAC rosettes has not previously been reported in the literature. In the present study it was determined that most erythrocyte profiles were closely apposed to the lymphocyte surface, forming broad zones of contact. Some of these broad zones appeared smooth, whereas others appeared highly irregular, resulting in deformation of the erythrocyte surface in some instances. The smooth broad zones of contact frequently extending over several microns of membrane surface have been previously reported for EAC rosettes.[17-19] In only one report were irregular broad zones shown on an EAC rosette.[17] Notably, this lymphocyte revealed the presence of inclusions which might represent parallel tubular arrays if examined at a higher magnification. The extreme deformation of the erythrocytes noted with the PTA lymphocyte EAC rosettes has not, however, been previously shown on EAC rosettes.[17-19]

This deformation, characterized by an infolding or puckering of the erythrocytes in the region of contact, is commonly seen in monocyte anti-D,Rh(+) rosettes,[20] monocyte anti-O,Rh(−) rosettes,[15] monocyte EAC rosettes,[21] lymphocyte anti-D,Rh(+) rosettes,[22] and lymphocyte anti-O, Rh(−) rosettes.[15] Lay et al [21] pointed out the ultrastructural similarity between rosettes obtained in a system involving erythrocytes sensitized by these 7S antibodies and in those in which binding is mediated by complement components. Unfortunately, their electron micrographs were only made of monocyte EAC rosettes and polymorphonuclear leukocyte (PML) EAC rosettes. Since a non-PTA lymphocyte cannot truly be identified in thin-sectioned material, one cannot determine whether the differences noted between the PTA lymphocyte EAC rosettes reported here and those EAC rosettes reported in the literature are a result of different subpopulations of lymphocytes being examined or are simply a result of

different planes of section. We have indeed shown by examining different PTA lymphocyte EAC rosettes that there is great variability in the mode of attachment of erythrocytes. Serial sections of the same rosettes also show different kinds of attachment present on the same erythrocyte. Answers to this question will have to await the isolation of a pure population of PTA lymphocytes.

Broad zones of contact, whether smooth or irregular, appear to be characteristic of EAC rosettes in general and have not been reported to occur with E rosettes.[19,23,24] Although one of our PTA lymphocyte EAC rosette profiles appeared E-rosette-like (Figure 2), one of the nine surrounding erythrocytes showed an irregular, broad zone of contact that extended over several microns of membrane surface. This scalloped surface appearance has not been described in any E rosette preparation.

The complement receptor on the PTA lymphocyte, when activated, appears to behave in a manner similar to that of the monocyte and PML by increasing surface activity. PTA lymphocytes are even capable of "eating," provided they are fed a "palatable" organism. This phagocytic activity may be a "vestigial" function left over from a precursor cell type before cell specialization in the immune system evolved. Although lymphocytes have some lysosomes in their cytoplasm, it is highly unlikely that they contribute significantly to the killing of bacteria. It is possible that C3 receptors on PTA lymphocytes function to localize antigen or induce blast transformation and division, as suggested for B lymphocytes.[25] Since it is possible that PTA lymphocytes may belong to the third population of lymphocytes in the peripheral blood, a complement receptor may be important in activating its "killer" machinery.[26]

References

1. Ross GD: Surface markers of B and T cells. Recent technical developments reveal a heterogeneity of lymphocyte subpopulations. Arch Pathol Lab Med 1977, 101:337–341
2. Payne CM, Glasser L: The effect of steroids on peripheral blood lymphocytes containing parallel tubular arrays. Am J Pathol 1978, 92:611–618
3. Payne CM, Jones JF, Sieber OF Jr, Fulginiti VA: Parallel tubular arrays in severe combined immunodeficiency disease: An ultrastructural study of peripheral blood lymphocytes. Blood 1977, 50:55–64
4. McKenna RW, Parkin J, Gajl-Peczalska KJ, Kersey JH, Brunning RD: Ultrastructural, cytochemical, and membrane surface marker characteristics of the atypical lymphocytes in infectious mononucleosis. Blood 1977, 50:505–515
5. Carlo JR, Nagle RB, Shin ML: The nature of the receptor for complement (C3b) in the human renal glomerulus. Am J Clin Pathol 1978, 69:486–493
6. Gelfand JA, Fauci AS, Green I, Frank MM: A simple method for the determination of complement receptor-bearing mononuclear cells. J Immunol 1976, 116:595–599
7. Stjernswärd J, Jondal M, Vánky F, Wigzell H, Sealy R: Lymphopenia and change in distribution for human B and T lymphocytes in peripheral blood induced by irradiation of mammary carcinoma. Lancet 1972, 1:1352–1356

8. Kabat EA, Mayer MM: Experimental Immunochemistry. Second edition. Springfield, Ill, Charles C. Thomas, Publishers, 1961
9. Payne CM, Satterfield VF: A simple procedure for the preparation of rosetted cells for electron microscopy. (In press)
10. Spurr AR: A low-viscosity epoxy resin embedding medium for electron microscopy. J Ultrastruct Res 1969, 26:31–43
11. Breton-Gorius J, Reyes F, Duhamel G, Najman A, Gorin NC: Megakaryoblastic acute leukemia: Identification by the ultrastructural demonstration of platelet peroxidase. Blood 1978, 51:45–60
12. Wardlaw AC, Pillemer L: The properdin system and immunity: V. The bactericidal activity of the properdin system. J Exp Med 1956, 103:553–575
13. Trepel F, Waubke R, Begemann H: Phagocytose durch lymphocyten. Klin Wochensch 1966, 44:256–261
14. Elson CJ, Bradley J, Howells RE: The mechanism of rosette formation between Rh(D)-positive erythrocytes and peripheral blood lymphocytes from Rh isoimmunized individuals: The role of surface micro-projections. Immunol 1972, 22:1075–1086
15. Reyes F, Le Go A, Delrieu F, Bach JF: Ultrastructure of cells binding immunoglobulin-coated erythrocytes in rheumatoid arthritis. Clin Exp Immunol 1974, 17:533–546
16. Anderson WA, Trantalis J, Kang Y-H: Ultrastructural localization of endogenous mammary gland peroxidase during lactogenesis in the rat: Results after tannic acid-formaldehyde-glutaraldehyde fixation. J Histochem Cytochem 1975, 23:295–302
17. Douglas SD, Huber H: Electron microscopic studies of human monocyte and lymphocyte interaction with immunoglobulin- and complement-coated erythrocytes. Exp Cell Res 1972, 70:161–172
18. Chen L-T, Eden A, Nussenzweig V, Weiss L: Electron microscopic study of the lymphocytes capable of binding antigen-antibody-complement complexes. Cell Immunol 1972, 4:279–288
19. Bentwich Z, Douglas SD, Siegal FP, Kunkel HG: Human lymphocyte-sheep erythrocyte rosette formation: Some characteristics of the interaction. Clin Immunol Immunopathol 1973, 1:511–522
20. Lo Buglio AF, Cotran RS, Jandl JH: Red cells coated with immunoglobulin G: Binding and sphering by mononuclear cells in man. Science 1967, 158:1582–1585
21. Lay WH, Nussenzweig V: Receptors for complement on leukocytes. J Exp Med 1968, 128:991–1009
22. Abramson N, Lo Buglio AF, Jandl JH, Cotran RS: The interaction between human monocytes and red cells: Binding characteristics. J Exp Med 1970, 132:1191–1206
23. Kataoka K, Minowada J, Pressman D: Electron microscope study on human lymphocyte-sheep erythrocyte rosettes. J Natl Cancer Inst 1975, 55:1323–1325
24. Galey FR, Prchal JT, Amromin GD, Jhúrani Y: Letter to the editor. N Engl J Med 1974, 290:690
25. Nussenzweig V, Bianco C, Dukor P, Eden A: Receptors for C3 on B Lymphocytes: Possible Role in the Immune Response. Edited by B Amos. New York, Academic Press, 1971, pp 71–82
26. Van Boxel JA, Paul WE, Frank MM, Green I: Antibody-dependent lymphoid cell-mediated cytotoxicity: Role of lymphocytes bearing a receptor for complement. J Immunol 1973, 110:1027–1036

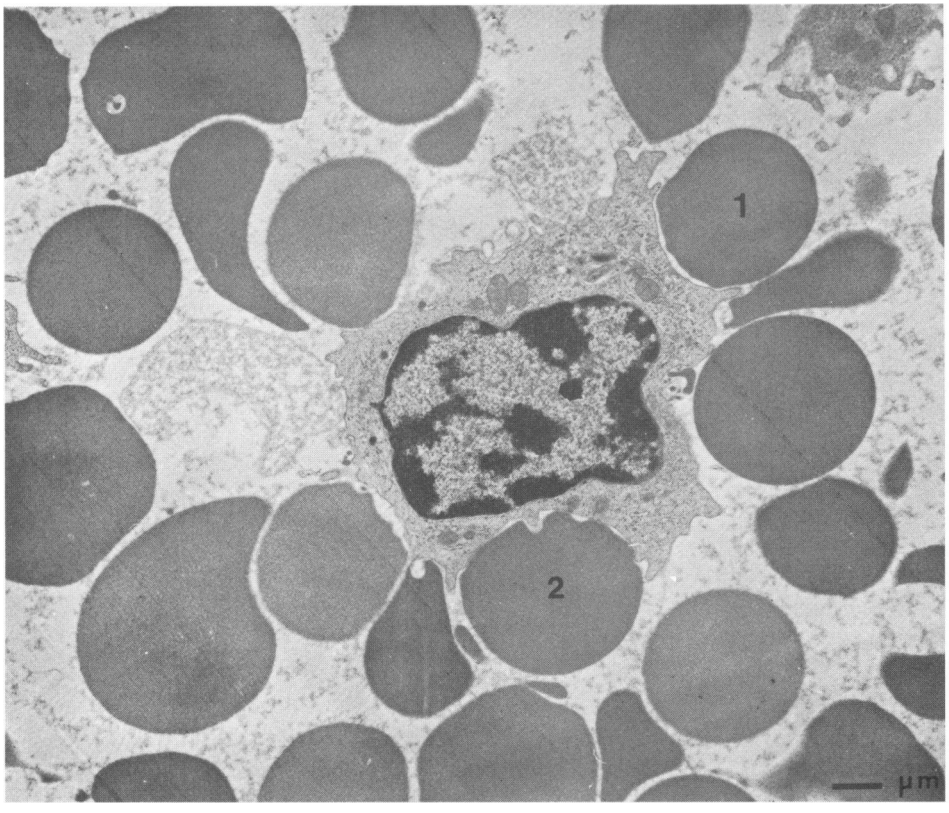
Acknowledgments

The authors wish to thank Dr. Jack Layton (Head, Department of Pathology) for his invaluable support, Ms. Virginia Satterfield for excellent technical assistance, and Ms. Sherry Fogleman for typing the manuscript.

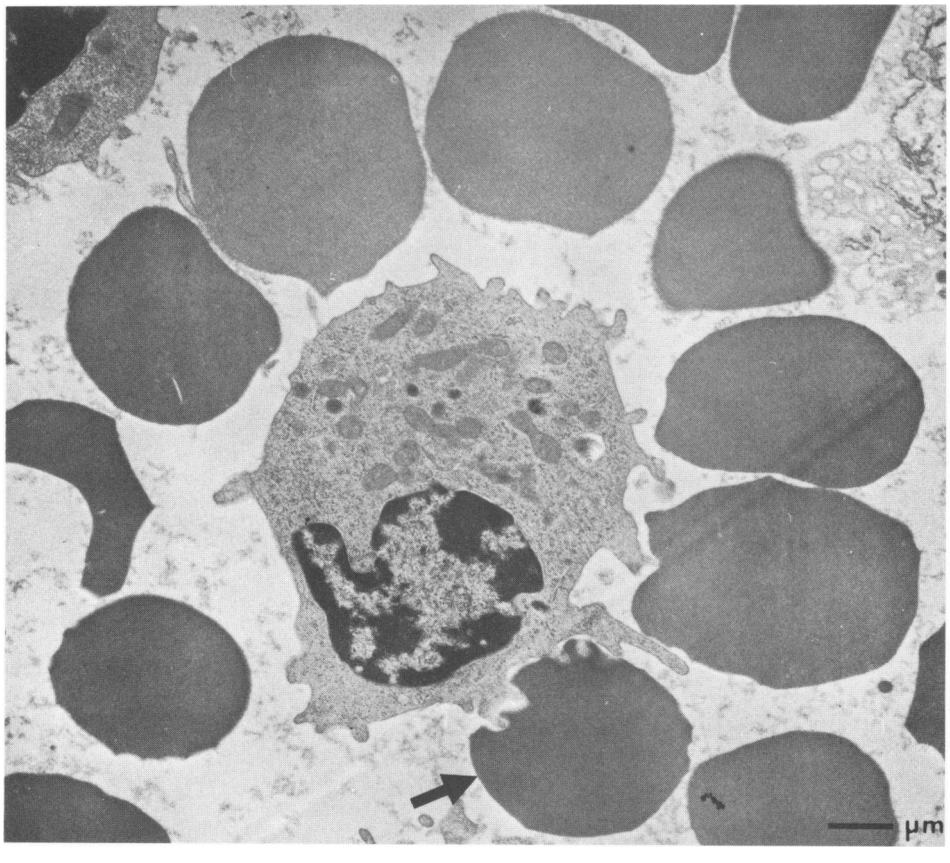
[*Illustrations follow*]

Figure 1—Low-power electron micrograph of a PTA lymphocyte EAC rosette from a man. Most of the attached erythrocytes are closely apposed to the lymphocyte surface. Erythrocyte 1 shows a smooth broad area of attachment, which extends over several microns of membrane surface. Erythrocyte 2 shows a more irregular broad area of attachment resulting in infolding of the erythrocyte surface. (×6600)

Figure 2—Low-power electron micrograph of a rare PTA lymphocyte EAC rosette from a man in which most of the attachment sites are out of the plane of section and the erythrocyte profiles appear to be further removed from the lymphocyte surface. Note the one erythrocyte (*arrow*) that shows an irregular, broad zone of contact extending over several microns of membrane surface. The erythrocyte surface appears deeply indented in four distinct areas by the lymphocyte's surface microvilli. (×8800)

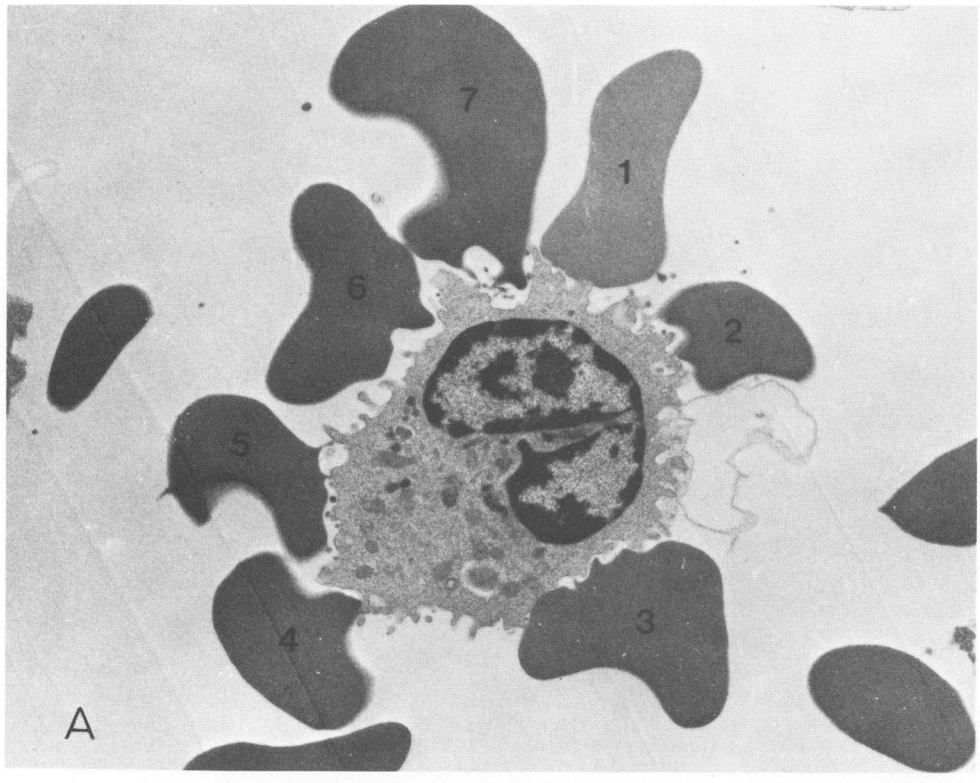


1



2

Figure 3—Composite electron micrographs comparing attachment sites in serial sections taken from the same PTA lymphocyte EAC rosette from a woman. The corresponding erythrocytes in both micrographs have been numbered. The surface microvilli of the PTA lymphocyte in the region of Erythrocytes 1, 2, and 7 in the bottom micrograph are much more numerous, and Erythrocytes 2 and 7 show more deformation of their surface membrane, as compared with the top micrograph. The left attachment area of Erythrocyte 3 in the upper micrograph shows a broader zone of adherence than in the bottom micrograph. Erythrocyte 5 in the upper micrograph shows greater deformation than in the lower micrograph, in which the attachment sites are out of the plane of section. (×6100)


7
1
6
2
5
3
4
A

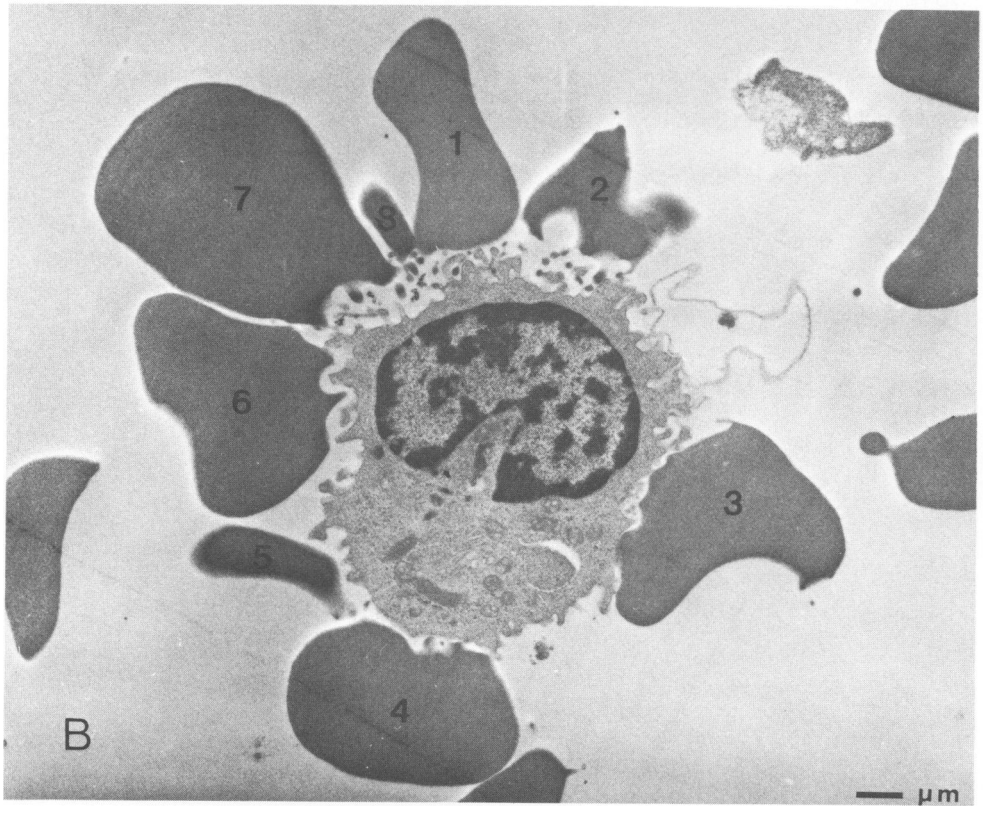
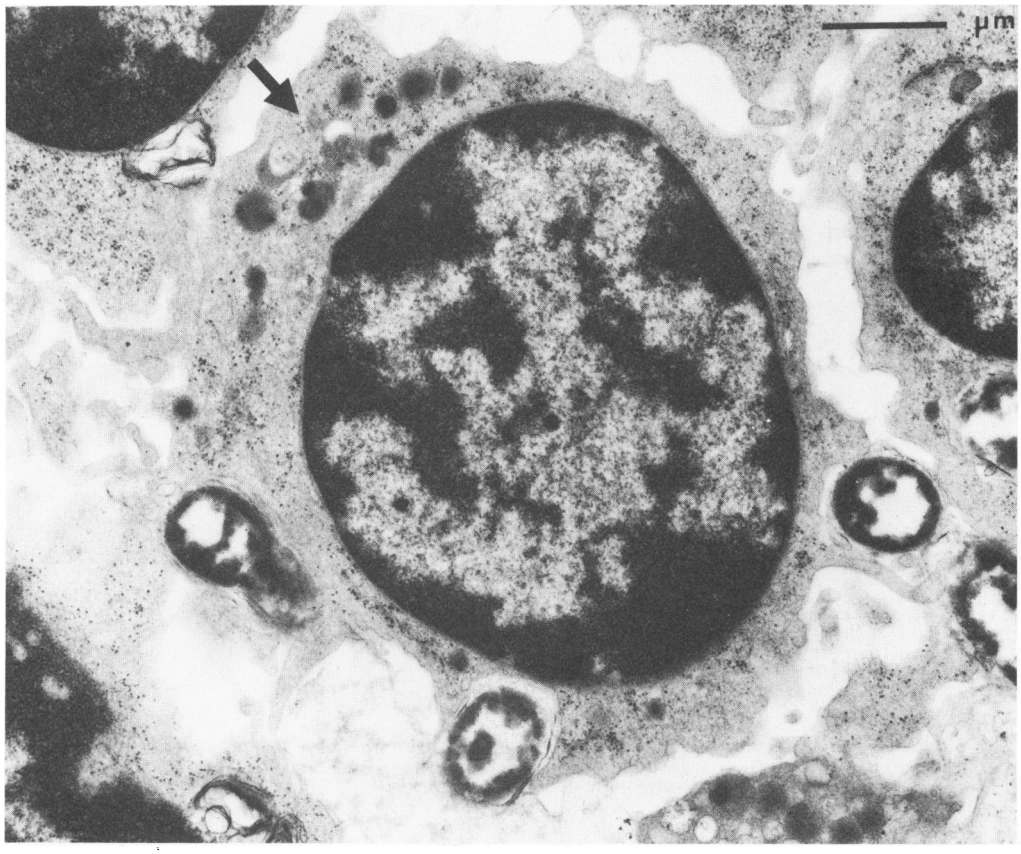
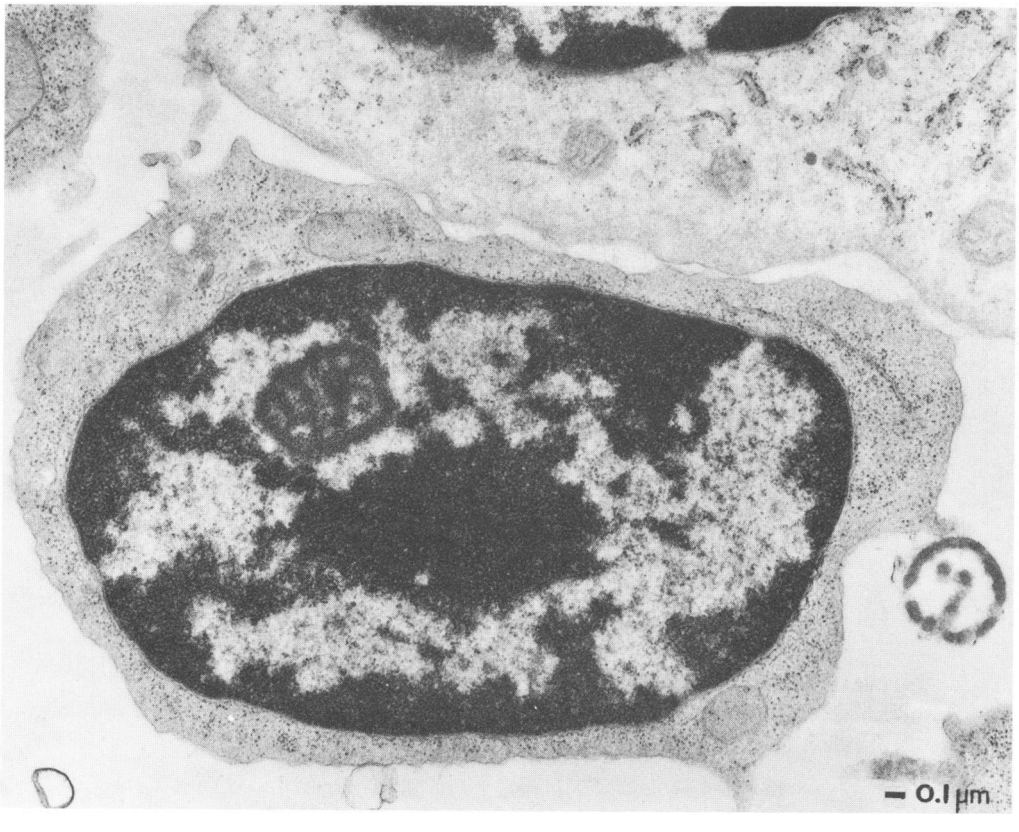

1
7
2
6
3
5
4
B
µm

Figure 4—Electron micrograph of a PTA lymphocyte FBC rosette from a man. Three *Salmonella typhi* organisms are found in depressions of the lymphocyte surface. The bacteria are partially surrounded by pseudopodal extensions of the PTA lymphocyte. The *arrow* points to several inclusions that at higher magnification were identified as parallel tubular arrays. (×16,300)

Figure 5—Electron micrograph of a lymphocyte from an FB rosette preparation obtained from a man. One *Salmonella typhi* organism can be seen touching a microvillous projection from the lymphocyte surface. (×21,800)



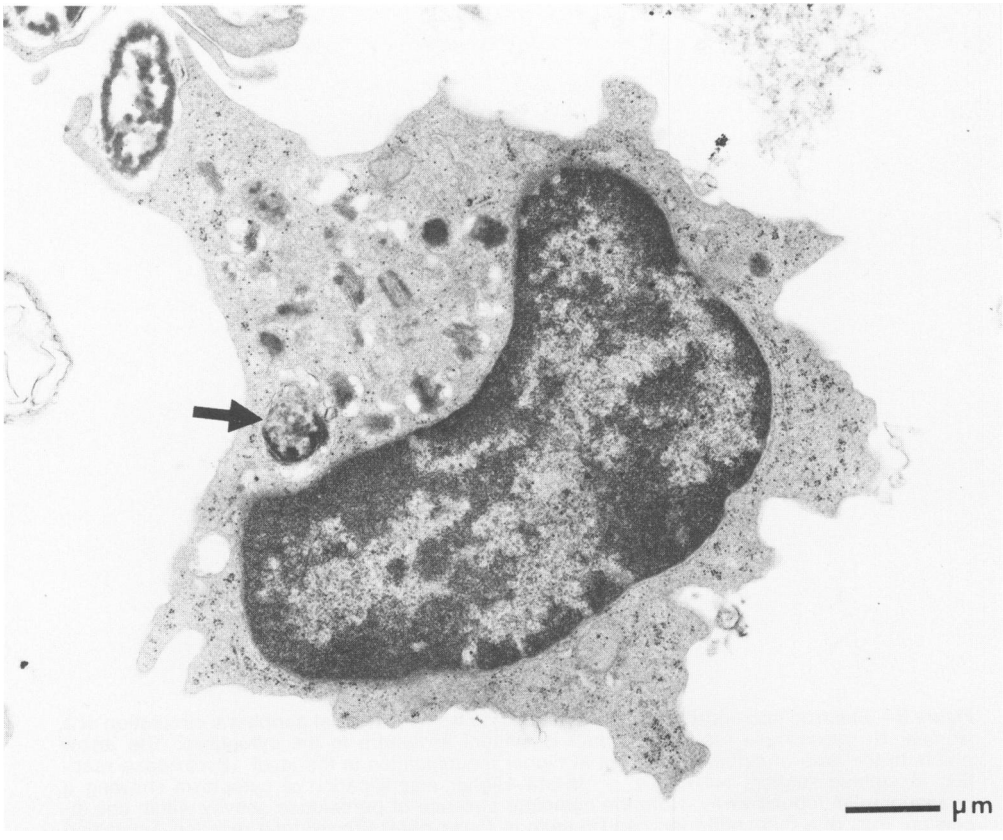
4



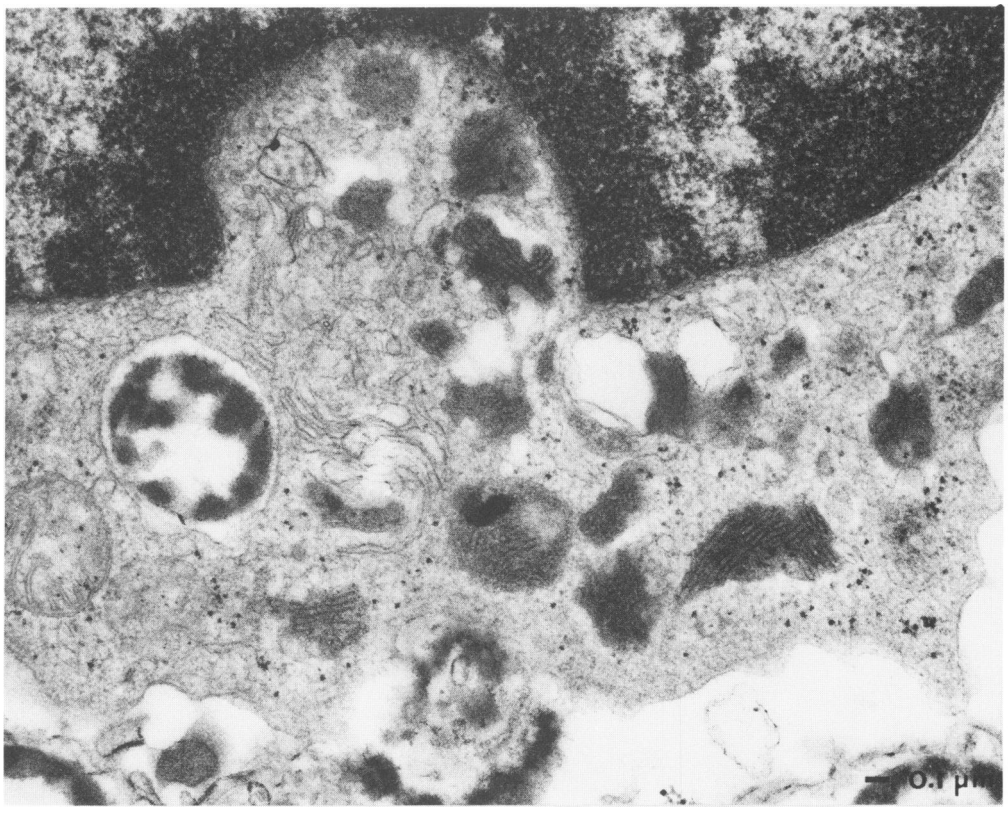
5

Figure 6—Electron micrograph of a PTA lymphocyte from a FBC rosette preparation (obtained from a man) in the process of phagocytizing a bacterium. One organism has already been phagocytized (*arrow*). (×12,500)

Figure 7—High-power electron micrograph of a PTA lymphocyte that has phagocytized a bacterium. Numerous parallel tubular arrays can be seen in the cytoplasm near the "hof" region of the nucleus. (×32,200)



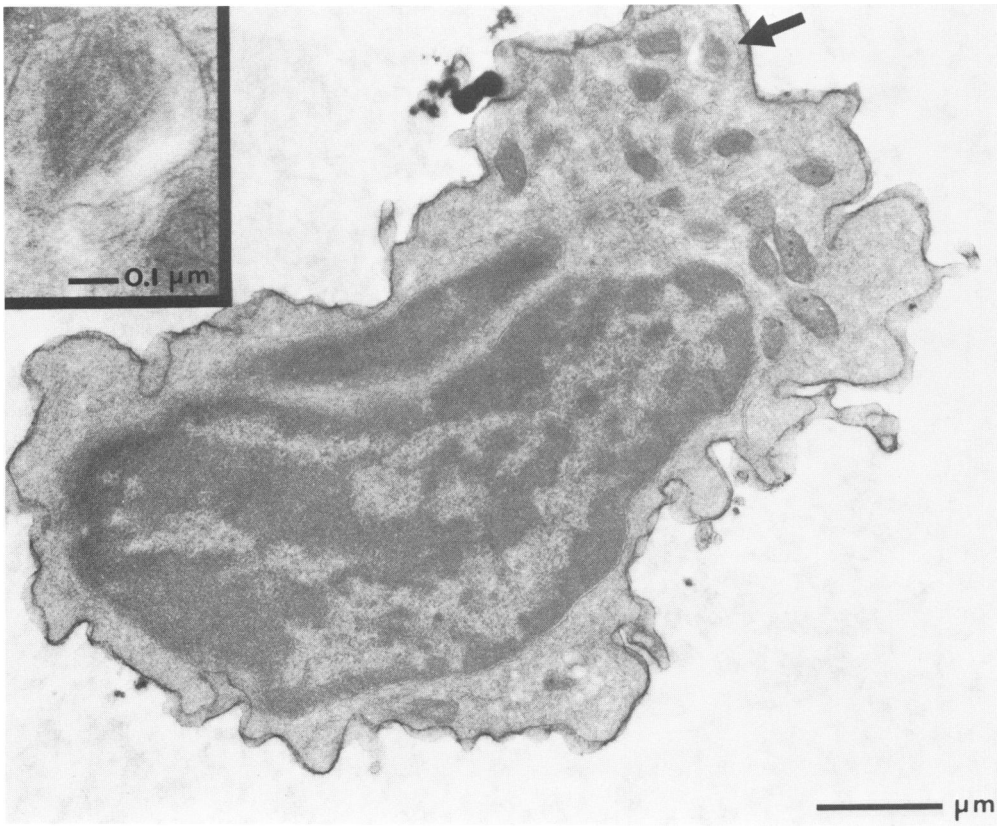
6



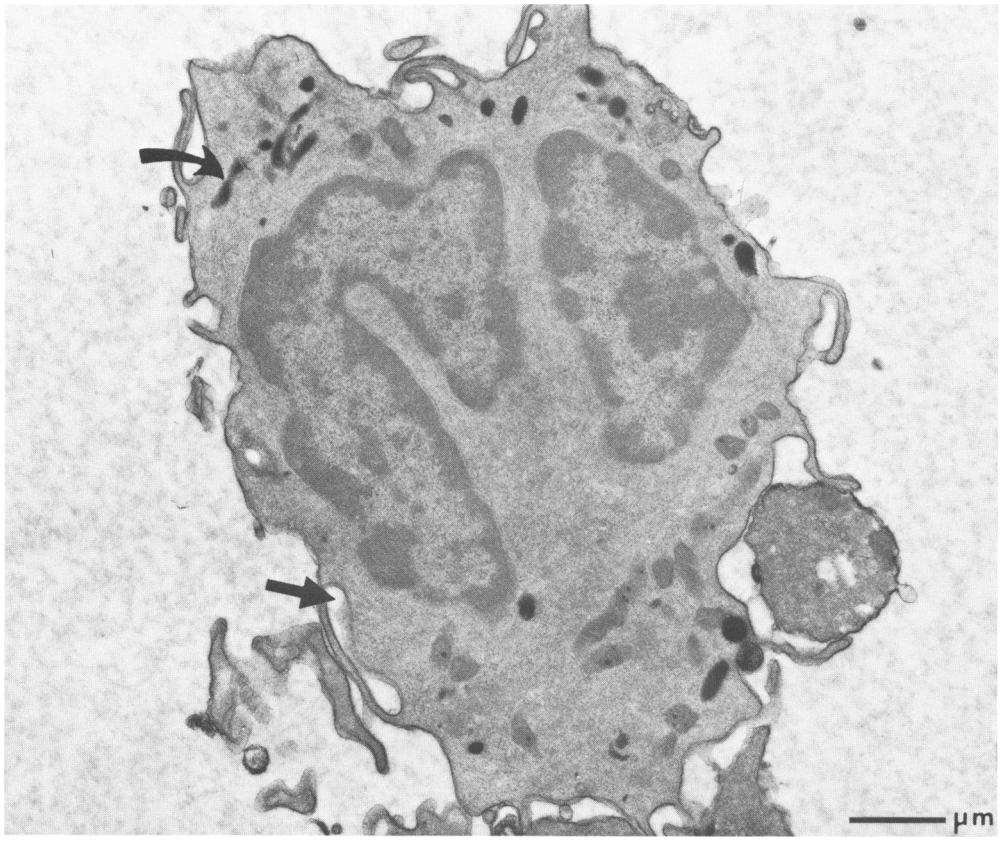
7

Figure 8—Electron micrograph of a PTA lymphocyte from the normal peripheral circulation of a woman. No peroxidase reaction product is evident anywhere in the cytoplasm. The *arrow* points to the area of cytoplasm shown at higher magnification in the inset. (Peroxidase reaction, unstained section, ×16,500) **Inset**—Higher magnification of cytoplasm showing a typical parallel tubular array. Note the complete absence of peroxidase activity within this inclusion. Part of a mitochondrion can be seen in lower right. (Peroxidase reaction, unstained section, ×65,800)

Figure 9—Electron micrograph of a monocyte from the normal peripheral circulation of a woman. Numerous peroxidase-positive granules are present in the cytoplasm, some of which have a typical dumbbell-shaped appearance (*curved arrow*). Another feature that identifies this cell as a monocyte is the presence of large pinocytotic vacuoles (*straight arrow*). (Peroxidase reaction, unstained section, ×12,800)



8



9

[*End of Article*]