MICROPAPILLAE

A Surface Specialization of Human Leukocytes

C. C. CLAWSON and ROBERT A. GOOD. From the Department of Pediatrics, University of Minnesota, Minneapolis, Minnesota 55455

The monocyte, the blood-borne representative of the reticulo-endothelial system (1), has several distinguishing functional attributes related to its cell surface which are dependent upon the presence of divalent cations (Ca^{++} and/or Mg^{++}). Among these are: phagocytic capacity, glass adhesion, surface binding of complement, and adhesion to other cells such as lymphocytes (rosette formation) or target cells in an immune cytolytic system. The following studies have defined a specialization of the cell surface which appears to be especially characteristic of the circulating monocyte. These surface organelles are small, lens-shaped projections or *micropapillae* (2) which are dependent for their preservation on the presence of physiologic levels of Ca^{++} and/or Mg^{++} and may actually bind significant quantities of these divalent cations.

METHODS

Normal human blood containing heparin (~10 units/ml) was diluted with dextran 75, 6% in saline (1 ml/5 ml of blood), and the erythrocytes were sedimented for 60 min, leaving a leukocyte-rich plasma supernate. The cell suspension was decanted into siliconized tubes and centrifuged at 300 *g* for 10 min. This procedure brought the bulk of all cell

types, including platelets, into a loosely packed pellet at the bottom. The supernate was decanted from the cell pellet and replaced by the fixative solution. A variety of standard and modified procedures for fixation were employed. These included: (*a*) 1% OsO_4 buffered to pH 7.4, (*b*) 1% fresh glutaraldehyde (Polyscience Corp., Evanston, Ill., 8%) in buffer, (*c*) 1% OsO_4 followed by 1% glutaraldehyde, (*d*) 1% glutaraldehyde followed by 1% OsO_4, (*e*) combined 1% OsO_4 and 1% glutaraldehyde, (*f*) 0.1% glutaraldehyde in Hanks' balanced salt solution (HBSS) (Gibco, Grand Island, N. Y.) pH 7.4 at 37°C for 10 min followed by 1% glutaraldehyde in HBSS at 37°C for 15 min, then 1% OsO_4 and 0.5% glutaraldehyde in HBSS for 60 min at ~4°C. In addition to standard HBSS, phosphate buffer, Tyrode's and White's saline were each tried for several specimens. Heparin and dextran were each eliminated from the preparation of some specimens, without influencing the appearance of the micropapillae. Regardless of the fixation employed, dehydration was accomplished with 10 min changes of 30, 50, 70, and 95% ethanols and three 10 min changes of absolute ethanol. The cells were embedded in Epon 812 (Shell Chemical Co., N. Y.) by standard procedures. Sections were cut with glass or diamond knives on a Sorvall MT2 (Ivan Sorvall, Inc., Norwalk, Conn.) or LKB Ultrotome. 1 μ sections were routinely prepared and stained with basic fuchsin, azure II-methylene blue (3). Grey-to-silver sections were collected on naked copper grids and examined in a Philips 200 electron microscope.

Several grids were prepared with sections from each specimen block. One grid was stained with saturated uranyl acetate (pH 3.8) and 1% lead citrate by standard methods. Another grid was left unstained. A third grid was stained with saturated aqueous uranyl acetate brought to pH 1.8–2 with HCl (acid–uranyl acetate).

In some experiments examination of the monocytes was facilitated by separation with the flotation technique of Bennett and Cohn (4). Leukocyte-rich plasma was obtained as above and centrifuged at 400 *g* for 10 min. The plasma was drained from the cell pellet and replaced by 27% bovine serum albumin (Pentex Biochemical, Kankakee, Ill.) in HBSS. The cells were resuspended, transferred to polyallomer tubes, and centrifuged at 2400 *g* for 40 min at 12°C. This brought the granulocytes, residual erythrocytes, and many lymphocytes to the bottom, while monocytes, some lymphocytes, and platelets formed a surface pellicle. This pellicle was removed with a Pasteur pipette and the cells were washed in two changes of HBSS with heparin and prepared for electron microscopy as above.

Paired specimens of concentrated monocytes were washed at 37°C for 10 min in Ca^{++}/Mg^{++}-free HBSS, 0.1% ethylenediaminetetraacetate (EDTA) in HBSS, or 0.38% Na citrate in HBSS. One specimen from each pair was then incubated 10 min at 37°C in HBSS with 5 mEq Ca^{++} per liter and 3 mEq Mg^{++} per liter and subsequently fixed for electron microscopy in fixatives containing the same levels of these ions. The second of the pair was handled in an identical manner but in Ca^{++}/Mg^{++}-free solutions. These specimens were coded and examined as unknowns.

RESULTS

Erythrocytes, neutrophils, small and large lymphocytes, monocytes, eosinophils, basophils, and platelets examined in sections with routine lead citrate and uranyl acetate staining displayed fine morphology consistent with published reports. At high magnification the monocyte surfaces frequently appeared to have a more granular, uneven distribution of densely staining material than other leukocytes, erythrocytes, or platelets. When unstained or acid–uranyl acetate–stained sections (discussed below) were examined, the granularity was found to be due in large part to the presence of previously undescribed surface structures (Figs. 1–3). These structures were electron-opaque, lens-shaped projections or micropapillae which were 200–300 A in diameter and 50–100 A thick with an outer radius of 150–200 A. The margins of the micropapillae appeared to be contiguous with the plasma membrane and the micropapillae were distributed randomly over the cell surface. It was estimated that a single large monocyte might have in excess of 30,000 micropapillae. They did not appear to be associated with pinocytotic vesicles or any cytoplasmic structure underlying the plasma membrane. Vacuoles in a more superficial location often contained micropapillae, while those located deep within the cell did not. The membranes of other organelles—mitochondria, endoplasmic reticulum, Golgi apparatus, and nucleus—revealed no structures resembling the micropapillae.

While the micropapillae were consistently present on cells which were readily identifiable as monocytes, occurrence of this feature varied in other cell types. Erythrocyte and platelet surfaces consistently lacked them. The great majority of lymphocyte sections failed to reveal any micropapillae; however, infrequently a few sections were found in which micropapillae were localized in one small region of the cell periphery. These micropapillae were on larger lymphocytes which displayed more folding and projections of the membrane in the same region where the micro-

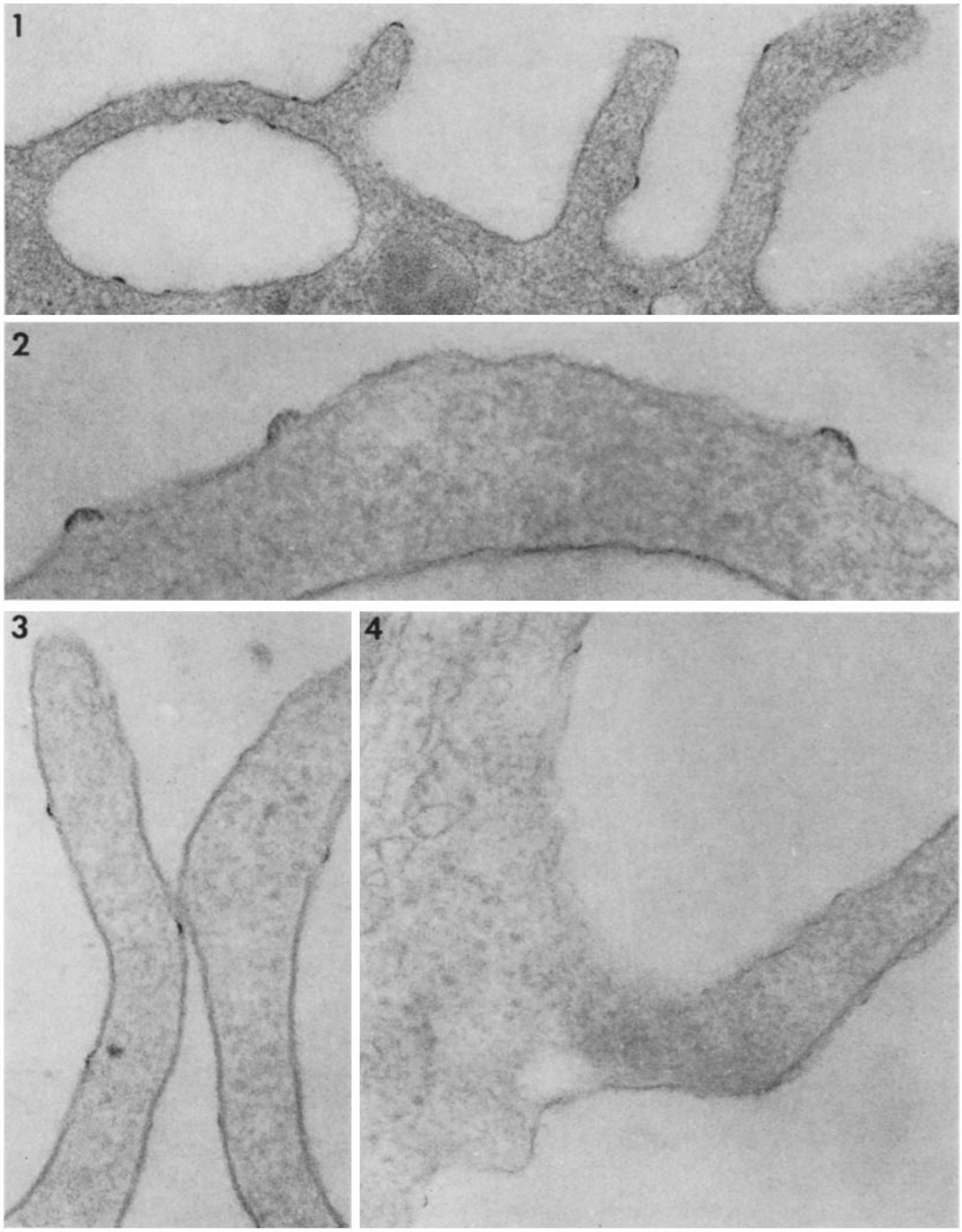

FIGURE 1 Surface of a normal human monocyte stained with uranyl acetate at pH 2 for 1 min (acid–uranyl acetate). Micropapillae are seen at the plasma membrane as small, densely staining, lens-shaped projections scattered over the cell surface. × 86,000.

FIGURE 2 Micropapillae on one of the microvilli of an eosinophil, Acid–uranyl acetate staining. × 186,000.

FIGURE 3 Microvilli of a monocyte with several densely staining micropapillae. Acid–uranyl acetate staining. × 86,000.

FIGURE 4 Neutrophil surface after treatment with EDTA followed by incubation in HBSS with normal levels of calcium and magnesium. Here the staining is the same as for cells not treated with EDTA. Similar cells treated with EDTA but without replacement of the divalent cations showed no staining of the micropapillae with acid–uranyl acetate. × 86,000.

papillae were found. The eosinophils and basophils usually had several micropapillae in any one section, but never in the profusion seen on the monocytes. Neutrophils were the least consistent of all cell types examined. They usually had numerous micropapillae in any given section; however, occasional neutrophil sections were found on which none could be identified. It was our impression that the micropapillae of neutrophils were on the average somewhat smaller and less electron opaque than those of the monocytes.

Nearly two dozen modifications of the preparative procedures were employed for the more than 50 specimens in which micropapillae have been found. The identification of these structures depends on both shape and staining which enhances their density over that of the adjacent membrane. The inclusion of glutaraldehyde and osmium tetroxide in some combination, and the presence of Ca^{++} and Mg^{++} in the fixative were required to preserve both structure and stainability of the micropapillae. Removal of Ca^{++} and Mg^{++} from the cells by washes with Ca^{++} /Mg^{++}–free HBSS, EDTA, or citrate all resulted in loss of clearly stainable and recognizable micropapillae. If, after these washes, the cells were restored to Ca^{++} /Mg^{++}–containing media and fixative solutions, the micropapillae were again visible and stainable with acid–uranyl acetate (Fig. 4).

The micropapillae fixed with osmium tetroxied had an electron opacity greater than that of adjacent membranes in otherwise unstained sections. This density could be removed along with other osmium staining by treatment with 2% H_2O_2 for 30 min. If the H_2O_2-treated sections were then stained with uranyl acetate, the contrast of the specimen, including the micropapillae, was restored. At the usual acidity of saturated aqueous uranyl acetate, ~pH 3.8, there was generally uniform staining of all cell structures, including the micropapillae. If, however, the acidity of the uranyl acetate was increased to pH 2.0, a selective staining of the micropapillae was obtained, enhancing their contrast over that of the more lightly staining adjacent membranes and other cell structures. Phosphotungstic acid at pH 1.5 failed to stain the micropapillae.

DISCUSSION

Two lines of evidence are presented which indicate the association of divalent cations with the micropapillae. The first is the requirement for Ca^{++} and/ or Mg^{++} in the cell media for their preservation. The second is the demonstration of differential staining with acid–uranyl acetate.

When uranyl acetate is dissolved to saturation in distilled water, the solution usually has a pH of 3.5–4.0. At this pH the uranyl acetate, a weak electrolyte, is mostly nonionized. That uranyl ion, UO_2^{++}, which is released is hydrolyzed to a complex with the general formula $UO_2(OUO_2)^{++}_{n+1}$ (5). Only at a pH below about 2.5 is UO_2^{++} present as the predominate ion (6). The uranyl ion complexes at cell surfaces with carboxyl and phosphoryl groups, but not with sulfhydryl groups, in competition with other cations (7). Rothstein (7) states that binding affinities of Ca^{++} and Mg^{++} for groups on the cell surface are 300 times greater than those of Na^{++} or K^{+} and that UO_2^{++} has more than 200 times the binding affinity of the calcium and magnesium ions. It follows, then, that if the pH of the uranyl acetate solution is lowered to 2.0, and cell sites of significant Ca^{++}/ Mg^{++} concentration are exposed to this solution, the UO_2^{++} will preferentially replace these ions and enhance the electron opacity of the site. The observations reported here indicate that this is the case for the micropapillae of leukocytes.

The results of this investigation may have special significance for the problems of leukocyte aggregation and adhesion to glass, processes which are dependent upon the presence of divalent cation. Cell surfaces brought into close proximity stabilize at a distance which just balances the attractive and repulsive forces at the plasma membranes. Curtis (8) applied the theoretical formulations of Verwey and Overbeek (9), and predicted that cells will stabilize at a distance of 100–200 A. This is consistent with the usual electron microscopic observations and would preclude any significant binding by direct chemical bonds, including calcium bridges. Therefore, Curtis (10) suggested that where divalent cations promote cell adhesiveness it may be through a reduction in net negative surface charge. This would decrease the repulsive force, and in turn the stabilization distance, resulting in greater opportunity for chemical bonding. However, Armstrong (11) was unable to show a direct correlation between reduction of negative surface charge by different divalent cations and the influence of these ions on cell aggregation. He proposed that the discrepancy might be explained if the cells had specialized

surface regions which are locally influenced by divalent cations. Lesseps (12) had previously pointed out that the repulsive force is directly proportional to the local radius of the interacting surfaces. Therefore, if the radius is reduced to less than about 1000 A, the stabilization distance would be reduced to ~5 A, or the order of magnitude of the theoretical calcium bridge. Electron microscopic studies have demonstrated that cells come in contact first at low-radius surface projections such as undulations or tips of microvilli; however, until the present study no specialized surface structures had been found. The micropapillae are cell surface specializations which are divalent cation–dependent, and have radii which fall below 1000 A. Whether they, in fact, are involved in cell adhesion remains to be demonstrated. Studies are now in progress to explore this problem.

We have made preliminary observations which indicate that as the monocyte transforms into a macrophage in cell culture its micropapillae increase appreciably in number. Thus these organelles may be an important part of the adaptive response of mononuclear phagocytes which plays a significant role in host defense mechanisms.

SUMMARY

Monocytes of normal human blood possess surface specializations, micropapillae, which are especially characteristic of this cell type. Other leukocytes have micropapillae, but less consistently or in smaller numbers than the monocyte. Micropapillae are lens-shaped projections of the plasma membrane which are 200–300 A in diameter and 50–100 A thick. They are dependent upon the presence of physiologic levels of divalent cation for their preservation. They are selectively stained with uranyl ions, probably because of the tendency for this ion to replace Ca^{++} and Mg^{++} at cell surface binding sites. Their function is unknown.

Dr. Clawson is a Faculty Research Associate of the American Cancer Society. Dr. Good is American Legion Memorial Heart Research Professor of Pediatrics and Microbiology.

This work was supported by the United States Public Health Service (AI-05153, AI-08677, AI-00798, NB-02042), The National Foundation—March of Dimes, The American Cancer Society, The Minnesota Division of the American Cancer Society, and the Minnesota Chapter of the Arthritis Foundation.

The authors acknowledge with sincere appreciation the technical assistance of Miss Jonalyn Kunce and Mrs. Patricia Mottaz.

Received for publication 5 June 1970, and in revised form 14 September 1970.

REFERENCES

1. COHN, Z. A. 1968. The structure and function of monocytes and macrophages. *Advan. Immunol.* **9**:163.
2. CLAWSON, C. C. 1969. Micropapillae on the plasma membranes of human leukocytes. *J. Cell Biol.* **43**(2, Pt. 2):23a. (Abstr.)
3. GHIDONI, J. J., M. M. CAMPBELL, J. G. ADAMS, H. THOMAS, and E. E. RAMOS. 1968. A new multicolored staining procedure for one-micron sections of epoxy embedments. Proceedings of the 26th Annual Meeting, Electron Microscopy Society of America. Claitor's Publishing Division, Baton Rouge, La. 240.
4. BENNETT, W. E., and Z. A. COHN. 1966. The isolation and selected properties of blood monocytes. *J. Exp. Med.* **123**:145.
5. AHRLAND, S., S. HIETANEN, and L. G. SILLEN. 1954. Studies on the hydrolysis of metal ions. X. The hydrolysis of the uranyl ion, UO_2^{++}. *Acta Chem. Scand.* **8**:1907.
6. AHRLAND, S. 1949. On the complex chemistry of the uranyl ion. I. The hydrolysis of the six-valent uranium in aqueous solution. *Acta Chem. Scand.* **3**:374.
7. ROTHSTEIN, A. 1962. Functional implications of interactions of extracellular ions with ligands of the cell membrane. *Circulation.* **26**:1189.
8. CURTIS, A. S. G. 1960. Cell contacts: Some physical considerations. *Amer. Natur.* **94**:37.
9. VERWEY, E. J., and J. OVERBEEK. 1948. Theory of the Stability of Lyophobic Colloids. Elsevier, N. V. Uitgevers Mij., Amsterdam.
10. CURTIS, A. S. G. 1962. Cell contact and adhesion. *Biol. Rev. (Cambridge).* **37**:82.
11. ARMSTRONG, P. B. 1968. On the role of metal cations in cellular adhesion: Effect on cell surface charge. *J. Exp. Zool.* **163**:99.
12. LESSEPS, R. J. 1963. Cell surface projections: Their role in the aggregation of embryonic chick cells as revealed by electron microscopy. *J. Exp. Zool.* **153**:171.