Electron Microscopic Study of Human Basophils

By DOROTHEA ZUCKER-FRANKLIN

THE RELATIONSHIP between mast cells and basophils has been a subject of debate since their discovery. Although both cell types have metachromatic granules which are thought to contain histamine\(^1\) and heparin,\(^3,5\) the granules of mast cells are not as water-soluble and are usually preserved in air-dried Giemsa-stained smears, whereas basophil granules always dissolve in water and are only preserved by absolute alcohol or aldehyde fixatives.\(^6\) This slight difference in solubility characteristics, as well as the greater availability of mast cells, may account for the fact that the ultrastructure of this cell type has been described by several investigators,\(^7\) whereas comparatively little is known about the fine structure of the human peripheral blood basophil. Thus, in the Electron Microscopic Atlas of Normal and Leukemic Human Blood by Low and Freeman,\(^11\) no illustration of the human basophil is shown, and Pease,\(^12\) in a study of marrow cells, commented that basophilic myelocytes are poorly fixed because of their watery consistency. The recent introduction of aldehyde fixatives into electron microscopy as well as the newer embedding agents have made a description of the ultrastructure of the basophil possible for the first time. The electron microscopic appearance of human basophil granules differs to some extent from that of human mast cell granules and is also at variance with the structure of basophil granules of other species reported to date.

MATERIALS AND METHODS

Peripheral blood buffy coats of normal subjects and human thoracic duct lymph were heparinized and collected as described previously.\(^13,14\) The specimens were fixed in phosphate-buffered 3 per cent glutaraldehyde at pH 7.4\(^15\) for 1 to 2 hours. Occasionally, 1 per cent acrolein\(^16\) was added to this fixative. To enhance contrast, the cells were placed into 2 per cent osmium tetroxide\(^17\) for 1 to 2 hours following glutaraldehyde fixation. Dehydration and embedding in Epon 812 were carried out by standard procedures.\(^18\) Thick sections (1 to 2 \(\mu\)) were prepared with an LKB ultrotome and examined with a phase microscope. Localization of basophils in thick sections was facilitated by staining with Toluidine Blue (Toluidine Blue 0, Fisher Scientific Co.). For this purpose, a filtered 1 per cent solution of the dye in distilled water was placed on the section. The microscope slide was heated for 30 to 60 seconds and washed. Despite the fact that the cells were embedded in epoxy resin, this stain imparted the specific purple red color\(^19\) to the granules. Areas containing basophils were selected for thin sectioning. These were stained with uranyl acetate\(^20\) and/or lead hydroxide\(^21\) and viewed with a Siemens Elmiskop I electron microscope at instrument magnifications ranging from 700 to 30,000.

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RESULTS

Basophils were readily found on low power survey because of the large size and osmiophilia of their granules (Figs. 1 and 2). Although eosinophilic granules are of similar size, the characteristic ultrastructure of the cell has been well established and presented no difficulty in differentiation (Compare Figs. 3 and 4). The distribution of nuclear chromatin in the multilobed nucleus of the basophil is identical to that described in other granulocytes (ref. 11 and Figs. 1-4). A nucleolus is only rarely encountered, and the loosely arranged " euchromatin" appears to be connected with the nuclear pores (Fig. 3). There is marked variation in the number, shape, size and internal structure of the basophil granules. They present round, oval and angular forms (Figs. 3 and 5-9) and range from 0.2 to 1.2 μ in their longest dimension. In general, the granules are distributed randomly throughout the cytoplasm, but when granulation is sparse, they appear to be located mostly in the periphery of the cell. Each granule is surrounded by a "unit membrane" and is partially or completely filled with particles of fairly uniform size. As a rule, a few rows of these particles are arranged along and concentric with the unit membrane, but in the central part of the granule, packing of the particles seems less organized (Figs. 7 and 8a,d). Although the size of the particles within each individual granule is uniform, their size varies from granule to granule within the same cell (Figs. 5 and 8-10). The largest intragranular particles measure about 200 Å in diameter; the smallest are difficult to resolve and tend to confer a homogeneous texture to the granule (Figs. 8c and 9). Periodic arrays, as those described by Fedorko and Hirsch in human mast cells,10 failed to be resolved in this latter type. Besides the particles, many granules show the development of concentric membranes resembling myelin figures (Figs. 8b, 8c and 9) which, on occasion, may be as elaborate as those reported in Kurloff bodies.23

In addition to the particles within the granules, the cytoplasm of basophils is replete with larger osmiophilic particles which conform in size, fixation and staining characteristics to glycogen24,25 (see Figs. 7-10). Although it has been reported that glycogen is delineated best when "stained" with lead,25 these particles are equally well resolved when uranyl acetate is the only contrast enhancing agent.

The circulating basophil has few, if any, ribosomes, and the Golgi zone is poorly developed. A few small profiles of rough-surfaced endoplasmic reticulum (ER) may be seen in basophils with sparse granulation (Fig. 6), but as a rule this organelle is not frequently encountered in this cell type. When the plane of section traverses the Golgi zone, a centriole is often present (Figs. 5, 6 and 11). Centriolar budding and related spindle tubules (Fig. 11) suggest the possibility that some of the circulating basophils are in prophase and able to undergo mitosis. The mitochondria are similar to those seen in eosinophils and always exceed the number present in neutrophils. The remainder of the cytoplasm shows a few profiles of smooth ER, a fairly large number of fibrils, which measure 100-150 Å in diameter and either crisscross the cytoplasm or appear in bundles (Figs. 6 and 11), and occasional microtubules (Fig. 12) which are similar to those reported in other cells.26
Fig. 1.—Phase micrograph of “thick section” (1–2 μ) of peripheral blood buffy coat. Arrows point to basophils. Granules appear purple when section is stained with Toluidine-blue. E: eosinophil. Mag. ×1000.

Fig. 2.—Survey electron micrograph of peripheral blood buffy coat. Arrow points to basophil. Note similarity of chromatin distribution in the nuclei of granulocytes. Mag. ×1400.

Fig. 3.—Peripheral blood basophil. Note multilobed nucleus (N) and variation in size, shape and composition of granules. (G). Arrows point to nuclear pores. Mag. ×11,000

Fig. 4.—Peripheral blood eosinophil. Ultrastructure of granules (G) is characteristic for this cell type. Chromatin distribution in nucleus (N) resembles that of basophil. Mag. ×7000.
Finally, a small percentage of basophils, which have a diameter of only 6 to 8 μ, present with highly variegated infoldings of the plasma membrane resulting in processes which extend parallel to the surface of the cell. (Fig. 9, inset) Though it is possible that cellular products could exit through such channels, material similar in appearance to the granule content has not been seen within them.
Fig. 6.—Detail of cell depicted in Fig. 5. Arrows point to cytoplasmic fibrils. A small profile of rough endoplasmic reticulum (ER) is seen. G: granules. M: mitochondria. N: part of nuclear lobe. C: centriole. Mag. ×61,000.

Fig. 7.—Magnification of area seen in inset of Fig. 5, showing characteristic architecture of human basophil granules. Extragranular particles (Gl) conform to descriptions of glycogen. Proximity of these particles to the granules suggests that they are related to chemical constituents of the granules. Mag. ×61,000.
Fig. 8.—Variety of basophil granules obtained from different cells.

A: Intrgranular particles do not entirely fill concave aspect of this bean-shaped granule (arrow). Note surrounding membrane. Origin: Human thoracic duct lymph. Mag. ×80,000.

B: Basophil granule depicting lamellar arrangement of intragranular membranes. Note that texture of area delimited by myelin membranes on left (asterisk) differs in appearance from remainder of granule. Mag. ×90,000.

C: Basophil granule with a more homogeneous texture. Note myelin membranes on top. Extragranular particles resemble structure of glycogen (GI). Mag. ×90,000.

D: Several granules with fine structure characteristic of human basophil. Note concentric arrangement of peripheral rows of intragranular particles. Extracellular particles presumed to be glycogen (GI). Mag. ×53,000.
Fig. 9.—Detail of peripheral blood basophil showing diversity of granules within the same cell. Granules 1 and 2 illustrate particles as well as "myelin" membranes. Granules 3 and 4 have a homogeneous architecture. Granule 5 depicts several units of concentric membranes surrounding areas within which no particles can be resolved. G1: glycogen. V: vacuoles. In some sections peripherally located "vacuoles" appear to be continuous with the extracellular space. Inset represents detail of the periphery of such a cell. Mag. ×63,000. Mag. of inset ×16,000.
Fig. 10.—Detail of basophil obtained from human thoracic duct lymph. Arrow points to granule which, in addition to typical particles, contains particles of larger size which resemble those seen in the cytoplasm and are alleged to be glycogen (G1 and Refs. 24, 25). This suggests either that glycogen is intimately associated with the granule content or that the particles represent a chemical substance other than glycogen which is related to granule products. Mag. ×54,000.

DISCUSSION

Until recently, attempts to analyze the ultrastructure of the human basophil have been thwarted by the inadequacy of fixatives and embedding materials. Most of the early descriptions failed to outline any detail in the granules, and it may be seriously doubted whether they dealt with normal circulating basophils at all. The only electron micrograph which suggested the ultrastructure described in this communication appeared in a review by Bessis and Thiery in which, in spite of the use of osmium tetroxide as a fixative and methacrylate as embedding agent, a fair amount of the structure of the granules had remained intact. On the other hand, efforts to preserve the basophils of several laboratory rodents met with more success, and several authors have described a skeletal network of lamellae within these cells. However, since most of the animals studied had a high level of circulating basophils and since, in most species, there appears to be a reciprocal relationship between the level of basophils and the number of tissue mast cells, one wonders whether such cells were not more comparable to human mast cells than to human basophils. Recent reports on human mast cells have shown lamellae, "scrolls," "whorls," crystals and particles (7–10), although these seem to differ from the lamellar network seen in the basophils of rodents as well as from the structures in human basophil granules described here. Never-
Fig. 11.—Detail of blood basophil showing centriole with spindle tubule formation (arrow). G: granule; M: mitochondrion. Mag. ×47,000.

Fig. 12.—Detail of blood basophil showing microtubules. The course of one microtubule is indicated by small arrows. Some small fibrils are also seen in the cytoplasm. G: granule. Mag. ×47,000.
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theless, on careful inspection of such published illustrations, a few granules, which resemble the ones shown in Figure 8, can always be found. Thus, even on the electron microscopic level, there is a certain degree of overlap in morphology of human mast cells and human basophils. It still remains to be established whether the ultrastructure correlates with the chemical constituents of the granules or whether it only reflects their functional state. Since "myelin" figures, such as the "scrolls" described in mast cells, consist of lipoprotein which, as a rule, is less water soluble than most mucopolysaccharides, the finding of fewer "scrolls" and other myelin figures in human basophils than in human mast cells could conceivably explain why the granules of the latter are somewhat less soluble. The reason for variations in appearance of the granules within the same basophil is also open to conjecture. In mast cells, it has been observed that the entire content of the granule discharges during degranulation. Although this mode of degranulation has not yet been demonstrated for basophils, the nature of the infoldings of the plasma membrane suggests that the same mechanism may be operative. This would make the secretory process per se unable to account for the variation in structure of the granules within the same cell, and would lead one to seek another explanation.

The presence of centrioles with spindle tubule formation was an unexpected finding which may indicate that the circulating basophil is not an end-stage cell as is the neutrophil. It would be of interest to know whether such cells return to the marrow or tissues to undergo mitosis or whether cell division may take place in the peripheral blood. In this connection, it is noteworthy that basophils like eosinophils are present in thoracic duct lymph, whereas few if any neutrophils enter the lymph provided that admixture with blood has not taken place. This makes it likely that the basophil is not only released into the circulation from the marrow, but that it may also enter the blood from other connective tissue sites. Mitotic figures, similar to those depicted, of basophilic myelocytes in guinea pig marrow have as yet not been encountered in the blood of normal subjects.

The presence of glycogen in basophils has been controversial, some authors contending that the PAS reaction is positive, others being unable to confirm this finding. Although it is very suggestive that the 250-300 Å cytoplasmic particles represent glycogen, electron microscopy cannot settle this question. Other materials may show similar structural and staining characteristics and a definitive identification must await fractionation studies. Since most of the particles appear in the vicinity of the granules (Figs. 7, 8c,d and 10), it would also be possible that they represent the heparin precursor, heparin monosulfuric ester, which gives a positive PAS reaction.

Lastly, it should be emphasized that the ultrastructure of the basophil granules described here is to a certain extent dependent on the type, concentration and pH of the fixative, as well as on other aspects of the procedures used in preparing the specimen. Since, however, under these conditions the results are consistent, the illustrations presented here may be helpful in identifying the basophil when similar electron microscopic studies are carried
out on normal human blood. They may also serve as a basis for future experimental work designed to elucidate the functional aspects of this cell.

SUMMARY

Electron microscope studies were carried out on human basophils obtained from peripheral blood and thoracic duct lymph. Delineation of the cells in thick sections was facilitated by staining with Toluidine Blue, which imparted the characteristic purple-red color to the granules. The cytoplasm contains many of the organelles seen in other cells, including a small Golgi apparatus, centrioles, mitochondria, fibrils and microtubules. Ribosomes and rough ER are rarely encountered. Basophil granules are surrounded by a unit membrane and contain particles which are uniform in size within the same granule but which vary in size in different granules within the same cell. Some granules reveal a homogeneous texture and/or "myelin" figures. The appearance of most basophil granules differs from the ultrastructure of human mast cell granules reported to date. It is suggested that the difference in fine structure may reflect a difference in the chemical constituents of the granules.

SUMMARIO IN INTERLINGUA

Studios per microscopia electronic esseva effectuate in basophilos human obtenite ab sanguine peripheric e ab le lympha de ducto thoracic. Le delineation del cellulas in crasse sectiones esseva facilitate per tincturation con blau toluidinic resultante in le characteristic coloration purpuro-rubie del granulos. Le cytoplasma contineva multes del organellas vidite in altere cellulas, incluse un micre apparato Colgi, centriolos, mitochondrios, fibrillas, e microtubulos. Ribosomas e crude reticulo endoplasmatic esseva incontrate rarmente. Le granulos basophilic esseva circumdate per un membrana unitan e contineva particulas uniforme in dimension intra le granulo individual sed variante in granulos differente intra le mesme cellula. Certe granulos revelava un textura homogenee e/o figuras de "myelina." Le apparentia del majoritate del granulos basophilic differeva ab le ultrastructura de human granulos mastocytic reportate usque al presente. Ex suggestionate que le differentia in le ultrastructura reflecte possibilemente un differentia in le constituentes chimic del granulos.

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REFERENCES

4. Behrens, M., and Taubert, M.: Der Nachweis von Heparin in den baso-
5. Martin, H., and Roka, L.: Zur Frage des Heparin-Gehaltes der Blutmast-
7. Stoeckenius, W.; Zur Feinstruktur der Granula menschlicher Gewebsmastzel-
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38. Wislocki, G. B., Rheingold, J. J., and Dempsey, E. W.: The occurrence of the periodic acid-Schiff reaction in various normal cells of blood and

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