HISTOCHEMISTRY OF THE CENTRIOLES AND CENTROSOMES OF THE LEUKEMIC CELLS FROM HUMAN MYELOBLASTIC LEUKEMIA

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Recent studies have provided considerable information concerning the morphology and fine structure of the centrioles and centrosome (4, 6, 12). Although little is known of the chemical composition of these structures, histochemical evidence indicates that they are composed of basic protein, ribonucleoprotein, and glycoprotein (7, 17); the centrosome or astrosome accumulates sulfhydryls during mitosis (13). It is the purpose of this study to examine the histochemical composition of the centrioles and centrosome observed in human leukemic myeloblasts during interphase.

Leukemic myeloblasts observed from two patients with acute myeloblastic leukemia exhibited exceptionally prominent centrosomes and readily identifiable centrioles. Usually, the positive recognition of centrioles either in normal or leukemic cells of the blood and bone marrow is difficult or impossible and cytoplasmic granules or mitochondria residing in close proximity to the centrosome may be mistaken for centrioles.

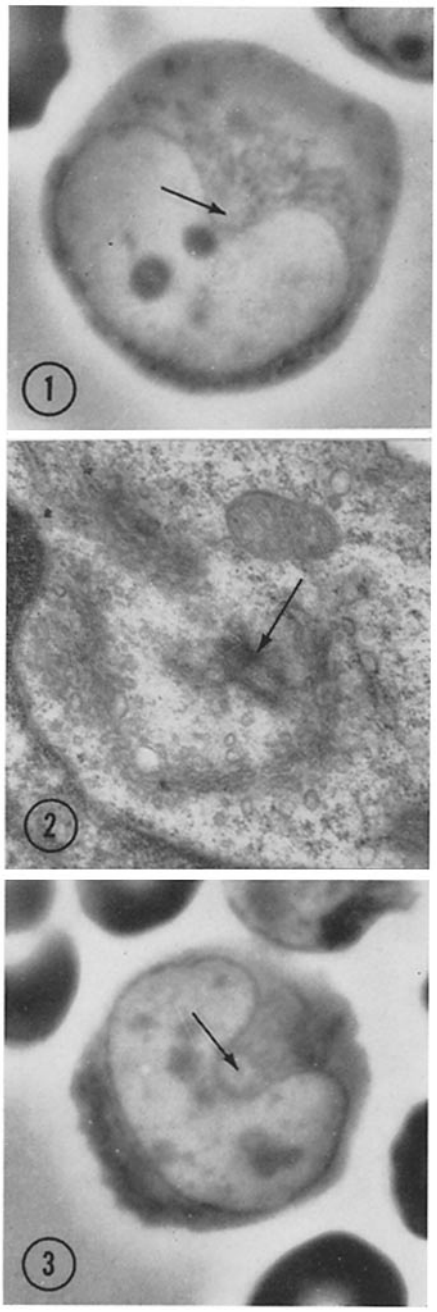
Vital and supravital (neutral red and Janus green) films were examined with brightfield and phase contrast microscopy. The following histochemical procedures were carried out on blood films after appropriate fixation: Wright's stain and ribonuclease digestion for pentosenucleoprotein (2); Fast green FCF at pH 2 for basic protein (16); Weiss, Tsou, and Seligman's method for protein-bound amino groups (18); Landing and Hall's method for histidine (14); Glenner's post coupled benzilidine method for indole derivatives (tryptophane) (10); Barrnett and Seligman's method for protein-bound sulfhydryl groups (3, 5), the periodic acid–Schiff method for glycoproteins and salivary digestion for glycogen (2), Sudan black B for lipids (2), naphthol AS-MX phosphate method for alkaline phosphatase (8), naphthol AS-D chloro-acetate method for esterase (15), Burstone's method for alanyl aminopeptidase (1, 9) and Graham's method for peroxidase activity (11). Blood samples obtained from one patient were fixed in buffered osmium tetroxide, embedded in methacrylate, and examined under the electron microscope.

Phase contrast microscopy revealed the centrosome to be optically less dense than the surrounding cytoplasm (Figs. 1, 3). Spherical mitochondria tended to be localized near the well circumscribed cytocentrum. In the living leukemic interphase cells, the centrioles, although slightly smaller than the mitochondria, exhibited similar optical density (Figs. 1, 3). Centrioles were observed to move slowly within the centrosomal zone. The centrioles and centrosome did not stain with either neutral red or Janus green in supravital films. Electron microscopy confirmed the presence of centrioles in the clear zone or centrosome located within the nuclear hof (Fig. 2). The centrosome tended to be surrounded by Golgi membranes and vesicles, contained few ribosomes, and possessed fewer small protein-like particles than the surrounding cytoplasm.

Histochemically, the centrioles exhibited slight basophilia removable by ribonuclease digestion. They revealed a weak periodic acid–Schiff (saliva-resistant) reaction, were slightly sudanophilic and Fast green–positive, colored very lightly following reactions for protein-bound amino groups and sulfhydryls, and gave negative reactions for histidine and indole derivatives as well as esterase, aminopeptidase, alkaline phosphatase, and peroxidase activity. The histochemical reactivity of the centrioles with Sudan black, Fast green, sulfhydryls, and protein-bound amino groups was less intense than the coloration of the mitochondria in these cells. The centrosome exhibited only a faint trace of basophilia (ribonuclease-labile) and appeared unstained with the other histochemical methods employed. Centrosomes observed in both normal and leukemic blood and bone marrow cells from other individuals exhibited similar

histochemical reactivity; however, as previously indicated, absolute recognition of centrioles in these cells has not been possible. It was frequently necessary to use phase microscopy to localize the centrosome and centrioles and change to bright-field microscopy in order to determine whether color was imparted to these structures by the histochemical reactions. Because of the minute size and faint coloration exhibited by the centrioles, photographic documentation of their histochemical reactivity was unsatisfactory.

The following points should be considered in the interpretation of weak or negative histochemical reactivity of the centrioles: (*a*) it may offer a crude index of the relative concentration or presence of a given substance within these bodies; (*b*) it may reflect lack of sensitivity and/or specificity of the method at this level of observation; (*c*) inability to color may be due to the physicochemical nature of these structures; and (*d*) perhaps coloration may result from non-specific background staining. Considerable experience at the cytological level with blood and bone marrow films, using the histochemical methods employed in this study, suggests that the faint colorations obtained represent positive reactions rather than non-specific coloration or binding. Negative histochemical reactivity, however, does not preclude the presence of the substances tested in either the centrosome or centrioles.



SUMMARY

These histochemical studies indicate that centrioles observed in the human leukemic interphase myeloblasts are composed of basic and sulfur-containing proteins associated with pentosenucleic acid, phospholipid, and polysaccharide. The centrosome of

FIGURE 1

Phase contrast microscopy of a living leukemic myeloblast showing numerous mitochondria in the deeply basophilic cytoplasm. Arrow indicates the clear spherical centrosome with visible centrioles located in the nuclear hof. × 2,600.

FIGURE 2

Electron micrograph showing the Golgi membranes surrounding the centrosome. Arrow indicates a centriole within the centrosome. Note the paucity of ribosomes and less dense small particles within the centrosome as compared with the surrounding cytoplasm; several Golgi vesicles also located within the centrosome. × 14,800.

FIGURE 3

Phase contrast microscopy of a living leukemic myeloblast with prominent centrosome and centrioles (arrow). × 2,600.

the leukemic myeloblasts possesses less structural protein and pentosenucleic acid than the centrioles and cytoplasmic matrix and appears to be devoid of stainable lipid, glycoprotein, sulfhydryls, and hydrolytic enzyme activity, in contrast with some of the observations reported by Stich on *Cyclops* eggs (17).

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