Ultrastructural Studies of the May-Hegglin Anomaly

By S. W. Jordan and W. E. Larsen

The inherited condition known as the May-Hegglin anomaly is characterized by the occurrence of crescent or spindle-shaped cytoplasmic inclusions containing ribose nucleic acid (RNA) in polymorphonuclear leukocytes in combination with platelet abnormalities. May (1909) noted the presence of distinctive basophilic, pyroninophilic patches in the cytoplasm of polymorphonuclear leukocytes of a healthy woman. Hegglin (1945) reported similar cytoplasmic inclusions in the polymorphonuclear leukocytes of a man and his two sons, in association with thrombocytopenia and giant platelets. A patient described by Leitner and co-workers (1954) had an acute illness suggesting a septicemia and the case could not be considered to be a genuine example of the May-Hegglin anomaly. Another case was described by Scholer and co-workers in 1960. The hereditary nature of the condition was clarified further by Petz and associates (1960), Oski and co-workers (1962), and Wassmuth et al. (1963). The findings of these investigators indicate that the May-Hegglin anomaly is inherited as an autosomal dominant trait. All patients so far described have characteristic inclusions in a large portion of their polymorphonuclear leukocytes. There is variable expression of the platelet abnormality. All patients have some giant platelets and some individuals show thrombocytopenia while others have a normal platelet count.

The majority of patients described are well, with no symptoms or disabilities ascribed to the abnormality of their leukocytes and platelets.

The purpose of the present report is to describe the ultrastructure of the leukocytes and platelets of a healthy woman who has the May-Hegglin anomaly, and to confirm the familial incidence of the affection.

Case Report

The patient, a 33-year-old Caucasian woman, was first seen at the University of Kansas Medical Center in October, 1962, for evaluation of generalized muscular and joint pain. The patient had been in good general health except for migratory arthritic pains, which were most marked in the knees at the time she was seen. There was a history of easy bruising since childhood. Physical examination revealed no joint deformity or limitation of motion, petechiae or ecchymoses. No organic basis for the arthritic pain was found and the patient did not exhibit significant hemorrhagic manifestations.

Laboratory Examinations. Hemoglobin, 13 Gm. per cent; hematocrit, 41.5 ml. per 100 ml.; white blood count, 5200/mm.³ with a differential of 61 per cent polymorphonuclear neutrophils, 7 per cent band forms, 1 per cent basophils, 18 per cent lymphocytes and 10 per cent monocytes. Platelet count was 38,000/mm.³ Repeat peripheral blood examina-

From the Department of Pathology and Oncology and the Department of Internal Medicine, University of Kansas Medical Center, Kansas City, Kansas.
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tion performed 1 week later was similar. Basophilic crescent and spindle shaped inclusions were observed in the cytoplasm of 97 per cent of polymorphonuclear leukocytes (figs. 1, 2). These inclusions were strongly pyroninophilic. Many giant platelets were present (fig. 1). Bone marrow aspirated from the sternum showed a normal number of megakaryocytes, many of which were lymphoid in type. Some of the megakaryocytes did not show platelet fragmentation, however many megakaryocytes contained giant platelet forms. Characteristic basophilic cytoplasmic inclusions were present in the cytoplasm of metamyelocytes, bands and mature polymorphonuclear leukocytes. Cytoplasmic inclusion bodies were seen in a rare myelocyte, but were not seen in myeloblasts nor in erythroid series cells.

Family Studies. Because of the reported familial incidence of the May-Hegglin anomaly, peripheral blood smears from the patient's mother, father, brother and only child, a son, were obtained. Typical basophilic inclusions were present in 6 per cent of the polymorphonuclear leukocytes from the patient's mother, and there were numerous giant platelets, although the total number of platelets appeared normal. The other relatives had normal peripheral blood smears.

Electron Microscopic Observations. Venous blood was collected from the propositus into ACID solution, centrifuged, and the buffy coat fixed in 0.6 per cent osmium tetroxide buffered to pH 7.4 with s-collidine. The material was dehydrated, embedded in epon 812 and 100 m sections were cut with a Porter-Blum ultramicrotome and glass knives, stained with lead or uranyl acetate and examined in RCA EMU 9G or JEM 5G electron microscopes.

RESULTS

Platelet clumps, neutrophilic and eosinophilic polymorphonuclear leukocytes, lymphocytes and monocytes were observed by electron microscopy. Lymphocyte and monocyte fine structure appeared normal and the ultrastructure of many polymorphonuclears did not seem unusual. About 20 per cent of the polymorphonuclear leukocytes had irregularly shaped cytoplasmic regions, which lacked specific granules and which have not, so far, been described in normal cells of this type.10,11 These regions corresponded in size and shape to the characteristic pyroninophilic cytoplasmic inclusions seen with the light microscope. The small proportion of polymorphonuclear cells showing inclusions by electron microscopy is striking when contrasted with the large percentage (97 per cent) noted by light microscopy. The explanation for this discrepancy probably is that a given section samples only a small part of the leukocyte, about 3 per cent of the total volume. The characteristic cytoplasmic patches seen by electron microscopy probably correspond to the basophilic inclusions seen with the light microscope because of similarity in size, shape, and location of these patches.

Ultrastructurally, the cytoplasmic patches are separated rather distinctly from the remainder of the cytoplasm (figs. 3, 4), and some of them are bounded partially by membranes of rough endoplasmic reticulum. They consist of small granules, 200 A. in diameter, probably glycogen12 in slightly higher concentration than in surrounding cytoplasm, amorphous homogeneous densities, and fine electron-dense fibrils approximately 50 A. in diameter and of variable lengths (figs. 5, 6, 7). Figure 8 shows several platelets. The average diameter of platelets was 4 µ, approximately twice the diameter of normal platelets prepared in a similar manner. Specific granules, mitochondria, and
Fig. 1.—Two neutrophilic polymorphonuclear leukocytes (NP) showing typical spindle shaped basophilic inclusions (arrows). Giant platelets (P) are also seen. Peripheral blood smear, Wright stain, Mag. x 1500.

Fig. 2.—Neutrophilic polymorphonuclear leukocyte showing pyroninophilia of a characteristic inclusion (I). Nucleus (N). Peripheral blood smear, methyl green-pyronine, phase contrast, green filter. Mag. x 3300.
Fig. 3.—Part of a polymorphonuclear leukocyte showing an inclusion (I), which lacks specific neutrophilic granules and which corresponds to inclusions seen by light microscopy. Nucleus (N), Erythrocyte (E). Electron micrograph. Mag. x 17,500.
Fig. 4.—Part of a polymorphonuclear leukocyte with inclusion (I) corresponding to the pyroninophilic inclusions seen by light microscopy. Mag. × 16,500.
Fig. 5.—View of characteristic inclusion in polymorphonuclear leukocyte. Note small granules, 200 Å. diameter, and fine fibrils (arrows) which are 50 Å. in diameter. Mag. x 45,000.

Fig. 6.—Inclusion in polymorphonuclear leukocyte showing small granules which probably represent glycogen, fine fibrils, probably RNA, and amorphous electron densities. Mitochondria (M). Mag. x 40,000.
Fig. 7.—Part of a polymorphonuclear leukocyte showing 200 Å diameter granules, 50 Å diameter filaments, and amorphous densities. Nucleus (N). Mag. x 75,000.

other granulomeric organelles appeared similar to those of normal platelets (fig. 9). Small regions composed of intricately folded membranes were seen in occasional platelets. The proportion of hyalomere and granulomere was variable, probably due to partial viscous metamorphosis of some platelets before fixation which resulted in central aggregation of granulomeric elements.

DISCUSSION

Beginning with the initial descriptions of the May-Hegglin anomaly in 1909 and 1945, 26 patients, including the present cases, have been reported. With the possible exception of 3 cases, no patients exhibited hemorrhagic manifestations or abnormalities of blood coagulation although minor changes in prothrombin consumption and other clotting factors have been reported. One of the cases reported by Oski and co-workers showed purpura, suggesting that patients with the May-Hegglin anomaly may have minor hemorrhagic phenomena.

The propositus reported here had the classic triad of the May-Hegglin anomaly: crescent shaped or spindle shaped, basophilic, pyroninophilic cytoplasmic patches in a high proportion of polymorphonuclear leukocytes (97 per cent), thrombocytopenia and giant platelets. The mother of the propositus had characteristic patches in a small proportion of polymorphonuclear leukocytes (6 per cent) and showed giant platelets with normal platelet number.
Fig. 8.—A platelet clump, showing platelets of twice the usual diameter, which other than for size, have a normal ultrastructural appearance. Mag. x 16,500.
Fig. 9.—Part of a platelet, showing apparently normal mitochondria, specific granules and vesicles. Small granules, 200 Å in diameter, appear similar in the cytoplasm of platelets, polymorphonuclear leukocytes, monocytes and lymphocytes. Mag. x 33,000.

The finding of a small proportion of affected polymorphonuclears in this patient indicates that the May-Hegglin anomaly may show considerable variability of genetic expression.

The characteristic pyroninophilic inclusions in polymorphonuclear leukocytes from the propositus corresponded in size, shape and location to the cytoplasmic areas seen ultrastructurally. They showed absence of specific granules, and consisted of small granules about 200 Å in diameter, fine fibrils which were 50 Å in diameter and amorphous densities. Since pyronine has been shown to stain RNA specifically, and because the staining disappears following exposure to ribonuclease, it follows that the characteristic inclusions contain RNA. The question then arises which of the components of the inclusions consists of RNA. The 200 Å granules are found diffusely in the cytoplasm of all varieties of leukocytes and platelets. The nature of these granules is unclear, but their ultrastructural appearance and distribution would suggest they represent glycogen. These granules stained intensely with lead, a characteristic of glycogen, but were poorly seen in sections which were unstained or in those stained with uranyl acetate. Thus it is unlikely they represent either ferritin molecules or ribosomes. In addition, the characteristic structure of ferritin was not demonstrated. Other more faintly staining granules were present in the cytoplasm of the leukocytes which were compatible with ribosomes. The 200 Å granules were present in only slightly higher concentration in the inclusions than in cytoplasm generally, which
also argues that they could not be responsible for the localized pyroninophilia. It would seem, therefore, that either or both of the remaining components of the characteristic inclusions may consist of RNA. Some of the fibrillar material had a diameter of 50 Å, which is about twice that of double stranded RNA in an extended form. Single strand fibrillar RNA, 10 to 12 Å in diameter, has been described by Warner and co-workers in association with ribosomal clusters from reticulocytes. It is suggested by these authors that the fibrillar RNA described by them is messenger RNA. In some micrographs of the presently reported material, it appears that much of the amorphous electron-dense material seen in the inclusions also has a fibrillar texture, so that much of the amorphous material may also consist of fibrils less than 50 Å in diameter, arranged in a tangled, random fashion.

It would seem, then, that the characteristic inclusions in polymorphonuclear leukocytes of patients affected with this hereditary anomaly of cell differentiation, consist of fibrillar RNA, amorphous electron densities, and glycogen macromolecules.

Platelets from the propositus are 2 times larger in diameter than normal. Rebuck and co-workers have measured the volume of May-Hegglin platelets in 1 patient and found them about 3 times normal in volume. Platelet specific granules, vesicles and mitochondria showed no recognizable abnormalities, perhaps not surprising considering that the ultrastructure of leukocytes also was within normal limits except for the characteristic inclusions. Central aggregation of granulomeric elements were manifest in many platelets, a change which occurs early in viscous metamorphosis. Later events in viscous metamorphosis, such as platelet fusion and dissolution with fibrin formation were prevented by the addition of ACD solution.

Platelets from persons with the May-Hegglin anomaly have been shown to undergo abnormal viscous metamorphosis when examined by a technic involving the mounting of whole, unsectioned platelets and examination with an electron microscope. This technic, because of the absence of anticoagulation, allowed observation of a more advanced stage of viscous metamorphosis than was seen in the presently reported material, so that the finding of apparently normal central aggregation of granulomeres seen in the sectioned material is not incompatible with abnormal viscous metamorphosis at a later stage.

It is interesting that leukocytic dysfunction at inflammatory sites in persons with the May-Hegglin anomaly has been reported. This consists of loss of the spindle shaped cytoplasmic inclusion by shedding, following migration of the polymorphonuclear leukocyte into the inflammatory site, and in seeming impairment of phagocyte activity. Similar phenomena were looked for in the present material processed in vitro. No evidence of extrusion or shedding of the characteristic inclusion was found.

**Summary**

One case of May-Hegglin's anomaly has been described with characteristic findings present also in the mother of the reported case. The propositus showed typical stigmata of the condition: characteristic crescentic, pyronino-
philic cytoplasmic patches in a high proportion of polymorphonuclear leukocytes, thrombocytopenia, and giant platelets. The patient’s mother, however, had characteristic patches in only 6 per cent of polymorphonuclear leukocytes and had giant platelets, but was not thrombocytopenic. This is the first patient reported to have such a small proportion of affected polymorphonuclear leukocytes, and suggests that there is variable expression of this genetically determined error of cell differentiation. Other family members did not show hematologic abnormalities.

The ultrastructure of leukocytes and platelets from the propositus was investigated, and it was found that:

1. No ultrastructural abnormalities were identified in the lymphocytes and monocytes.
2. Polymorphonuclear neutrophiles had irregular cytoplasmic areas which lacked specific granules and which contained small granules probably glycogen, 50 Å. diameter filaments, and amorphous densities.
3. The fibrils found in the characteristic patches probably consist of RNA.
4. Platelets have apparently normal ultrastructure, but are twice the normal diameter.

SUMMARIO IN INTERLINGUA

Es describite un caso de anormalitate de May-Hegglin, con constatationes characteristic presente etiam in le matre del patiente. Le patiente mesme exhibiva le stigmas typic del condition: characteristic crescentiforme, pyroninophilic maculas cytoplasmic in un alte proportion del leucocytos polymorphonucleari, thrombocytopenia, e plachettas gigante. Le matre del patiente, nonbstante, habeva maculas characteristic in solmente 6 pro cento del leucocytos polymorphonucleari. Illa habeva plachettas gigante sed illa non esseva thrombocytopenic. Isto es le prime subjecto con le condition in qui un si basse proportion del leucocytos polymorphonucleari esseva afficite. Isto suggere que il existe variabilitate del expression de iste geneticamente determinate error del differentiation cellular. Le altere examine membros del familia non monstrava anormalitates hematologic.

Le ultrastructura del leucocytos e plachettas del prime patiente esseva investigate. Le resultatos esseva le sequentes:

1. Nulle anormalitates ultrastructural esseva identificate in le lymphocytes e le monocytes.
2. Le neutrophilos polymorphonucleari habeva irregular areas cytoplasmatic disproviste de granulos specific, e continente micre granulos de (probablemente) glycogeno, filamentos de un diametro de 50 Å, e densitates amorphe.
3. Le fibrillas trovate in le maculas characteristic consiste probabilmente de acido ribonucleic.
4. Le ultrastructura del plachettas es apparentemente normal, sed lor diametro es augmentate per un factor de duo.

REFERENCES


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S. W. JORDAN and W. E. LARSEN

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