Intranuclear Crystalloids Associated With Abnormal Granules in Eosinophilic Leukocytes

By Richard T. Parmley, William M. Crist, Maryann Roper, Minoru Takagi, and Ronald L. Austin

Ultrastructural evaluation of eosinophilic leukocytes from a 2-yr-old asymptomatic girl with chronic benign neutropenia (CBN) revealed a variety of morphological abnormalities. All eosinophils obtained from blood and marrow specimens contained multiple microcrystalloids in most of the mature cytoplasmic granules. An increase in crystalloid-free, immature granules in late (bilobed nuclei) eosinophils suggested a delay in granule maturation. The eosinophil granules appeared to be of normal size and demonstrated normal acid phosphatase reactivity. Eosinophilic myelocytes contained abnormal cisternae of rough endoplasmic reticulum (RER) and lacked abundant elongated RER cisternae seen in normal cells. A few eosinophilic myelocytes in specimens of bone marrow from the child contained large intranuclear crystalloids measuring up to 3 μ in length. The intranuclear crystalloid contained a cubic lattice of dense material with a periodicity similar to that described for cytoplasmic crystalloids. The ultrastructural morphology of marrow neutrophils was normal, as described in other cases of CBN. Ultrastructural examination of blood eosinophils from the father demonstrated microcrystalloids in cytoplasmic granules identical to those seen in the child. The father was asymptomatic and had normal leukocyte counts. Thus, anomalous crystalloid granule genesis occurred in the father and daughter and was not necessarily associated with neutropenia or clinical symptomatology. This anomaly is associated with the accumulation of intranuclear crystalloid material in eosinophilic myelocytes, which do not appear to be released from the marrow compartment.

CASE REPORT

A 13-mo-old white female was referred to the Children’s Hospital for evaluation of neutropenia, which was first noted at 6 mo of age. She had had two episodes of skin infection at 6 and 10 mo of age that responded readily to oral antibiotic therapy. Physical examination was normal. There was no lymphadenopathy or organomegaly. The patient was in the 10th percentile for height and 5th percentile for weight. There was no history of serious infections, malignancy, or hematologic diseases in the family.

The child had been neutropenic since 6 mo of age (earlier blood counts were not performed). The total neutrophil count (band and segmented forms) varied between 0 and 693/cu mm, and the highest total eosinophil count was 240/cu mm. Monocyte, lymphocyte, and basophil counts were normal. The hemoglobin, hematocrit, red cell indices, platelet counts, and total white cell counts were normal. A bone marrow aspirate revealed a myeloid to erythroid ratio of 3.2:1, with decreased numbers of polymorphonuclear cells. Three percent eosinophils were noted in the marrow. Additional normal laboratory studies included erythrocyte sedimentation rate, normal. A bone marrow aspirate revealed a myeloid to erythroid ratio of 3.2:1, with decreased numbers of polymorphonuclear cells. Three percent eosinophils were noted in the marrow. Additional normal laboratory studies included erythrocyte sedimentation rate, serum immunoglobulins, serum complement, LE-cell preparation, antineutrophil antibodies were detected in the patient’s serum (test performed by Dr. Laurence Boxer, Indiana University School of Medicine, as described previously). An epinephrine stimulation test, performed by injecting the patient with 0.01 cc/kg of epinephrine (1:1000) and obtaining neutrophil counts over a 30-min period, did not result in a significant increase in the number of circulating neutrophils (maximum neutrophil count 432 at 30 min). The hydrocortisone stimulation test demonstrated a maximal rise in neutrophil number of 1470 at 3 hr after drug administration (3 mg/kg i.m.). Soft agar cultures of peripheral blood and bone marrow were performed according to a modified method of Robinson and Pike and demonstrated normal numbers of colony-forming cells (CFC) in marrow and blood. No inhibition of CFU generation was noted when the patient’s serum was added to cultures of her own or normal marrow or peripheral blood. Colony-stimulating activity obtained...
INTRANUCLEAR CRYSTALLOIDS IN EOSINOPHILS

from patient monocytes and serum, as described previously, was like that of monocytes from normal controls.

Now 25 mo old, the patient remains neutropenic; a repeat marrow aspirate at 20 mo was essentially the same as the initial specimen. Complete blood counts obtained from both parents were normal and showed no evidence of neutropenia.

MATERIALS AND METHODS

After informed consent, bone marrow aspirates and venous blood samples were obtained for routine light microscopic staining and ultrastructural studies when the patient was 13 and 20 mo of age. Heparinized venous blood samples were also obtained from the parents. For comparison, heparinized marrow samples from other children with chronic benign neutropenia were similarly processed as described previously. The heparinized specimens were centrifuged at 1500 g for 3 min to obtain a buffy coat, which was then immersed and finely minced in 3% glutaraldehyde 0.1 M cacodylate, pH 7.35, for 1 hr at 4°C. The specimens were then rinsed 3 times in 0.1 M cacodylate, 7% sucrose buffer, pH 7.35. In addition to processing specimens for routine morphology, some specimens were processed for cytochemistry. Acid phosphatase activity was demonstrated by incubating the specimens for 45 min at 37°C in a solution containing β-glycerol phosphate as a substrate at pH 5.0, as described by Barka and Anderson. All specimens were then postfixed in a 1% O2O4 solution in 0.1 M cacodylate for 1 hr at room temperature. The specimens were then routinely dehydrated in graded alcohols and propylene oxide and embedded in Spurr low viscosity medium. Thin sections (50-70 nm thick) of morphological preparations were counterstained with lead citrate and uranyl acetate, and examined in a Philips 300 electron microscope at an accelerating voltage of 60 kV.

RESULTS

Light microscopic review of the bone marrow aspirate from the child revealed several eosinophils in Wright’s stained smears. Immature specific granules of eosinophilic leukocytes appeared azurophilic, and mature granules appeared eosinophilic and often filled the cytoplasm, obscuring nuclear details. Single and coalesced eosinophilic granules were observed overlying or within the nucleus. It could not be clearly determined if some of this eosinophilic material actually represented intranuclear inclusions. Granules of neutrophilic and eosinophilic leukocytes demonstrated normal positive acid phosphatase, Sudan black B, and peroxidase staining. Several bone marrow macrophages (18%, n = 50) contained phagocyted neutrophils and neutrophil debris. Phagocytosis of eosinophils by marrow macrophages was not observed.

Ultrastructural examination of bone marrow and blood specimens revealed an abundance of neutrophils with normal morphology (Fig. 1). Neutrophil primary and secondary granule genesis appeared normal, and no evidence of excessive autophagy was observed. Several bone marrow macrophages contained neutrophils in various stages of degradation.

Blood and marrow eosinophils demonstrated a variety of ultrastructural abnormalities. Early eosinophils (promyelocyte-myeelocytes) contained a single nucleus with normally dispersed nuclear chromatin. Rare eosinophilic myelocytes (less than 0.5%) contained large dense intranuclear crystalloid inclusions that appeared symmetrical in shape (Figs. 1, 2, and 3). These inclusions measured up to 3.0 μm in length and 1.1 μm in width. In some planes of section, a cubic lattice of dense material was observed with a periodicity of 4 nm (Fig. 2, inset). The inclusions lacked acid phosphatase activity (Fig. 3). The rough endoplasmic reticulum of several eosinophilic myelocytes appeared as rounded profiles with occasional intracisternal precipitates (Figs. 1, 2, and 4). Late eosinophils contained normally lobulated nuclei with moderately condensed nuclear chromatin (Fig. 5).

Specific granules of eosinophilic leukocytes averaged 1 μm in diameter and could be divided into two broad categories; mature granules contained crystallloid material (Fig. 4), while immature granules lacked crystallloid material (Fig. 5), as seen in normal eosinophils. The specific granule matrix was densified by uranyl acetate staining, whereas the crystallloid material was relatively lucent as seen in normal specimens. The most striking abnormalities were observed in more mature crystallloid granules, which often contained numerous (more than 20) small crystalloids (Fig. 4). These abnormal granules were observed in all marrow and blood eosinophils and were the predominant type of granule in late eosinophils obtained from blood specimens of the child and her father (Table 1). These granules were only rarely observed in eosinophils from marrow and blood specimens obtained from the mother, normal volunteers, or other patients (n = 9) with CBN, 4 of which are included in the tabulated data (Table 1). A few granules in some eosinophils contained only 1 or 2 crystalloids similar to those seen in normal specimens. These normal appearing granules were most frequently seen in late (bilobed) eosinophils. Immature granules, lacking crystalloids, were prominent in early eosinophils and were also frequently observed in late eosinophils from the patient (Fig. 1), whereas these granules were almost always confined to early eosinophils in normal marrow specimens. The matrix of specific granules of eosinophils contained acid phosphatase activity similar to that observed in normal specimens.

DISCUSSION

The association of abnormal intranuclear and granule crystalloids in eosinophilic leukocytes observed in the present case has not been previously described. Although several nuclear and cytoplasmic abnormali-
**Fig. 1.** This low power electron micrograph includes an eosinophilic myelocyte (lower left), a late eosinophil (lower right) with a bilobed nucleus, and a neutrophilic myelocyte (above). The eosinophilic myelocyte contains an intranuclear crystalloid (enlarged in the inset) and several cytoplasmic granules containing microcrystalloids. The late eosinophil contains this latter abnormal granule type as well as an excessive number of immature granules lacking crystalloids (arrows). The neutrophilic myelocyte appears morphologically normal and contains electron dense primary granules (P) and smaller, less dense secondary granules (S). (x 13,400; inset x 27,900.)

*The specimens for Figs. 1–5 are from marrow samples of the child. The thin sections for Figs. 1, 2, 4, and 5 are counterstained with lead citrate and uranyl acetate, whereas that for Fig. 3 is not counterstained.*
Fig. 2. This eosinophilic myelocyte contains a large central nuclear crystalloid. The cytoplasm contains numerous granules with multiple microcrystalloids (enlarged in upper inset) and abnormal circular profiles of rough endoplasmic reticulum (RER). At high magnification, a cubic lattice of periodic material with 3-4 nm spacing can be seen at the thin edge of the intranuclear inclusion (lower inset). (x 19,700; upper inset x 39,400, lower inset x 197,000).

Fig. 3. The intranuclear crystalloid of this eosinophilic myelocyte lacks acid phosphatase reactivity. The condensed nuclear chromatin displays nonspecific lead deposition. Reaction product is present in the matrix of a specific granule (arrow). (x 12,000).

Fig. 4. Numerous fragmented crystalloids are present in the specific granules of this bilobed (nucleus not illustrated) eosinophil. A few cytoplasmic granules contain a single large crystalloid that appears normal. The relative electron lucency of the crystalloids in this preparation is more evident than in other figures. (x 24,000).
ties have been observed in leukemic\textsuperscript{14-18} and inflammatory\textsuperscript{6,7} eosinophils, these abnormalities have not included the presence of intranuclear inclusions. The cells containing these giant intranuclear crystalloids are not released from the marrow compartment and are presumably destroyed prematurely. The lack of associated clinical symptoms suggests a benign disorder; however, identification of this anomaly in other individuals and additional follow-up are required before the clinical significance can be fully assessed.

The presence of multiple microcrystalloids (often more than 20/granule) in the majority of specific granules of eosinophils has not been previously described. These microcrystalloids are occasionally...
observed in cytoplasmic granules; however, these INTRANUCLEAR CRYSTALLOIDS IN EOSINOPHILS

cytophiles from this patient indicates that eosinophils are associated with infectious complications. The lack of clearly related to the neutropenia in our patient. The

anomaly in a father and daughter suggests an autosomal dominant inheritance. Unfortunately, marrow specimens from the father could not be studied. Consequently, it could not be definitively determined if the nuclear abnormality observed in marrow eosinophils of the child are consistently related to the anomalous cytoplasmic granules.

The eosinophil abnormality does not appear to be clearly related to the neutropenia in our patient. The neutropenia noted in the child was similar to that we have observed in other patients with CBN, in that CFC and CSA were normal, maturation to segmented neutrophils in culture occurred, and no inhibitors to myelopoiesis were observed. Also, phagocytosis of neutrophils, but not eosinophils, by marrow macrophages was noted. Some cases of neutropenia have been associated with eosinophilia; however, these eosinophils have generally demonstrated normal morphological features or nonspecific changes associated with infectious complications. The lack of morphological abnormalities in neutrophilic leukocytes from this patient indicates that eosinophils are selectively affected by the presumed genetic lesion. Furthermore, the father, who also has the cytoplasmic granule anomaly, does not have neutropenia. Thus, it is probable that the anomaly of eosinophils and the neutropenia are occurring independently.

The crystalloid appearance of the intranuclear inclusion is similar to that described for specific granule crystalloids and Charcot-Leyden crystals. The observation of a cubic lattice with a 4-nm periodicity in the intranuclear crystalloid is similar to that described for normal crystalloids in cytoplasmic granules of eosinophils. Conceivably, the intranuclear crystalloid could reflect abnormal accumulation of material destined for the specific granules. This material could be synthesized by the nuclear envelope or by abnormally retained intranuclear ribosomes. A somewhat comparable situation has been described in myeloma cells where intranuclear inclusions resembling Russell bodies may be present; however, these inclusions differ from those in the present case in that they are membrane-bound.

The appearance of the microcrystalloids in maturing granules of early eosinophilic myelocytes suggests that the genesis of microcrystalloids occurs immediately after the immature (crystalloid-free) granule is formed and does not result from fragmentation of a large crystalloid in mature specific granules. The presence of some normal appearing specific granules in late (bilobed) eosinophils supports this hypothesis and suggests that microcrystalloids may coalesce to form larger crystalloids in specific granules. Furthermore, the presence of crystalloid-free (immature) granules in late eosinophils suggests an additional delay in specific granule genesis in eosinophils from our patient.

The abnormal rounded cisternae of rough endoplasmic reticulum (RER) in eosinophilic myelocytes and the presence of small dense inclusions in some of these cisternae suggest that a defect in protein synthesis or granule packaging may exist. This hypothesis is also consistent with the presence of intranuclear crystalloid material and abnormal cytoplasmic granule crystalloids. Conceivably, a defect in synthesis (qualitative or quantitative) and/or transport of a crystalloid precursor substance, possibly eosinophil basic protein, could result in formation of abnormal granule crystalloids and an accumulation of RER cisternal and intranuclear crystalloid precursor material. The latter could gain access to the nuclear compartment through nuclear pores or by abnormal intranuclear accumulation of ribosomal material on which the crystalloid material is synthesized.

Table 1. Granule-Crystalloid Number

<table>
<thead>
<tr>
<th>Specimen</th>
<th>n = 1</th>
<th>n = 2</th>
<th>n = 3</th>
<th>n = 4</th>
<th>n = 5 or Greater</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propositus marrow†</td>
<td>16</td>
<td>7</td>
<td>4</td>
<td>3</td>
<td>70</td>
</tr>
<tr>
<td>Control marrow‡</td>
<td>78</td>
<td>16</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Father's blood</td>
<td>18</td>
<td>12</td>
<td>7</td>
<td>3</td>
<td>60</td>
</tr>
<tr>
<td>Mother's blood</td>
<td>86</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

*A minimum of 200 crystalloid-containing granules were scored from at least 10 randomly selected eosinophils in each group. Eosinophil granules lacking crystalloids (n = 0) were not scored.
†Marrow and blood samples from the propositus demonstrated identical abnormalities in cytoplasmic granules.
‡The control consisted of micrographs of eosinophils obtained from marrow samples of 4 children with neutropenia.

The control consisted of micrographs of eosinophils obtained from marrow samples of 4 children with neutropenia. These results are similar to those observed in eosinophils from normal marrow specimens.
REFERENCES


Intranuclear crystalloids associated with abnormal granules in eosinophilic leukocytes

RT Parmley, WM Crist, M Roper, M Takagi and RL Austin