CONCISE REPORT

Deposition of Autofluorescent Eosinophil Granules in Pathologic Bone Marrow Biopsies

By Michael K. Samoszuk and Froilan P. Espinoza

Eosinophil granules are intensely autofluorescent when excited by green light. To determine if eosinophils degranulate in the bone marrows of patients with a variety of diseases, we used green light epifluorescence microscopy to examine deparaffinized and dezenkerized sections of 49 bone marrow core biopsies. In 14 of the biopsies, there was striking extracellular deposition of intensely autofluorescent eosinophil granules in addition to numerous intact eosinophils. Among the 14 specimens with extracellular autofluorescence were seven cases of leukemia, four cases of non-Hodgkin’s lymphoma, two cases of myelofibrosis, and one case of pancytopenia with eosinophilia. In the remaining 35 specimens, only intact eosinophils were identifiable. There was no extracellular autofluorescence in three normal marrows, four marrows from AIDS patients, or three biopsies from patients with idiopathic thrombocytopenic purpura (ITP). We conclude that green light epifluorescence microscopy identifies extracellular deposits of eosinophil granules in bone marrow biopsies of some neoplastic disorders and in diseases associated with reticulin fibrosis.

In bone marrow and peripheral blood, the granules of unstained human eosinophils are strikingly autofluorescent. The intensity and specificity of the autofluorescence allow eosinophils to be easily distinguished from other leukocytes by fluorescence microscopy or by cell-sorting.

Recent immunocytochemical studies indicate that eosinophils release granule proteins in soft tissues of patients with a variety of diseases. By contrast, eosinophil degranulation in bone marrow biopsies has been difficult to document because of the tissue denaturation produced by acidic fixation and decalcification.

In order to determine if eosinophil degranulation occurs in the bone marrows of patients with a variety of diseases, we examined unstained sections of deparaffinized and dezenkerized bone marrow core biopsies by epifluorescence microscopy. This report, therefore, compares the autofluorescence patterns of normal bone marrows, bone marrows with eosinophilia, and bone marrows from patients with neoplastic or myeloproliferative disorders.

MATERIALS AND METHODS

Tissues. Cases for study were retrieved from the Hematopathology Division files (1985-1987) at UCI Medical Center. Except as noted, only initial diagnostic or staging bone marrow biopsies of high quality (well-fixed; without crush artifact) were studied. The cases were grouped into the diagnostic categories listed in Table 1. For comparative purposes, we also studied three normal bone marrow biopsies and ten biopsies of patients with marked marrow eosinophilia (Hodgkin’s disease, three cases; AIDS, four cases; idiopathic thrombocytopenic purpura [ITP], three cases).

All patients were biopsied in the course of a diagnostic workup and were advised of the nature of the procedure and its attendant risks, in accordance with The University of California guidelines. The patients gave informed consent to the procedure and agreed that the tissue may be used for research, at the discretion of the pathologist. Because the study used only archival pathology tissue, it was reviewed and exempted from further approvals by the UCI Human Studies Review Committee.

The Zenkers-fixed, paraffin-embedded bone marrow biopsies were sectioned at 6-μm thickness, deparaffinized in xylene and graded alcohols, and dezenkerized in Lugol’s iodine solution. The sections were then coverslipped with PBS-glycerol (low-fluorescence) and examined within 24 hours.

Epifluorescence microscopy. The epifluorescence microscopy was performed on a Nikon Optiphot microscope (Nikon Inc, Garden City, NY) equipped with an EF-D episcopic-fluorescence attachment, UFX-II camera, and planapochromat objectives. A green light excitation filter (546 nm) was used together with a DM580 dichroic mirror and 580W eyepiece-side barrier filter. Illumination was provided by a high pressure mercury lamp (HBO100W/2).

Sections were examined independently by two observers, and the presence or absence of extracellular autofluorescent granules was recorded. For photomicrography, Kodak professional TMAX black and white film (ASA 400) was used.

RESULTS

Epifluorescence microscopy. The results of the visual examination of 49 cases are summarized in Table 1. In all instances, intact eosinophils were easily visualized (Fig 1); and in 14 of the cases, there was extracellular deposition of autofluorescent eosinophil granules. Except in two cases believed to contain coarse globules of slightly fluorescent lipofuscin, there was agreement between both observers on the presence or absence of extracellular autofluorescence. Older specimens had less intense fluorescence than more recent biopsies.

The extracellular autofluorescence was particularly extensive in the biopsies of chronic myelogenous leukemia (Fig 2). There was no consistent microanatomic localization of the granules; in some cases, a fine granularity was present in a patchy distribution while in others a paratrabecular deposition was noted. Extracellular deposits of autofluorescent granules were not identified by any of the normal bone

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Table 1. Results of Epifluorescent Microscopy of Bone Marrows

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Eosinophil Degranulation</th>
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<tbody>
<tr>
<td></td>
<td>Absent</td>
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<tr>
<td>Normal bone marrow (3)</td>
<td>3</td>
</tr>
<tr>
<td>Eosinophilic bone marrow in patients with Hodgkin’s disease (3)</td>
<td>3</td>
</tr>
<tr>
<td>Eosinophilic bone marrow in patients with AIDS (4)</td>
<td>4</td>
</tr>
<tr>
<td>Idiopathic thrombocytopenic purpura and marrow eosinophilia (3)</td>
<td>3</td>
</tr>
<tr>
<td>Reticulin fibrosis; pancytopenia (1)</td>
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</tr>
<tr>
<td>Marrow eosinophilia; pancytopenia (1)</td>
<td>0</td>
</tr>
<tr>
<td>Chronic myelogenous leukemia (3)</td>
<td>1</td>
</tr>
<tr>
<td>Acute myelogenous leukemia (10)</td>
<td>8</td>
</tr>
<tr>
<td>Acute myelomonocytic leukemia (1)</td>
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<tr>
<td>Acute lymphocytic leukemia (11)</td>
<td>9</td>
</tr>
<tr>
<td>Myelofibrosis (2)</td>
<td>1</td>
</tr>
<tr>
<td>Non-Hodgkin’s lymphoma in marrow (7)</td>
<td>3</td>
</tr>
<tr>
<td>Totals</td>
<td>35</td>
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*Including one case after chemotherapy. Extracellular deposits of a faintly fluorescent material were interpreted to be lipofuscin by one observer.

maws or in the hypereosinophilic biopsies from patients with Hodgkin’s disease, AIDS, or ITP.

DISCUSSION

The autofluorescence of 49 bone marrow biopsies representing a variety of diseases was studied. Extracellular deposition of autofluorescent granules was observed only in neoplastic conditions and in diseases associated with reticulin fibrosis, suggesting that the phenomenon is not an artifact of specimen processing. Because autofluorescence in marrows is relatively specific for eosinophil granules, we conclude that green light epifluorescence microscopy detects eosinophil degranulation in the bone marrow of patients with a variety of disorders.

The biological significance of this observation is not yet known, but the implications are intriguing. Eosinophil degranulation has been described in a number of disorders, including atopic dermatitis, onchocerciasis, eosinophilic endomyocardial disease, and Hodgkin’s lymphomas. All of these diseases are associated with some degree of fibrosis, and in this study, a similar association is suggested.

Moreover, the eosinophil granule is known to contain a wide variety of compounds with potent biological activities. We therefore speculate that degranulation of eosinophils in bone marrows with neoplasms could conceivably modulate the host inflammatory response to the neoplasm. Future studies correlating eosinophil degranulation with the clinical course will be of interest in determining the biological significance of the degranulation phenomenon in bone marrows.

REFERENCES


Fig 1. Photomicrograph of a bone marrow with eosinophilia but no evidence of extracellular autofluorescence. Intact eosinophils are brightly fluorescent and contain discrete cytoplasmic granules. Green light epifluorescence, with no counterstain. (Original magnification ×400; current magnification ×304.)

Fig 2. Photomicrograph of a bone marrow from a patient with chronic myelogenous leukemia. In addition to the numerous intact eosinophils, there is abundant deposition of finely granular autofluorescent material between the bone marrow cells. No counterstain. (Original magnification ×400; current magnification ×304.)


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