CORRESPONDENCE

Neutrophilic Nonspecific Esterase

To the Editor:

Gordon and Hubbard\(^1\) designated some cases as acute myelomonocytic leukemia because of leukocytes that stained with both the AS-D-chloroacetate esterase and the \(\alpha\)-naphthol acetate esterase (NSE) reactions. The dual reactivity is real; however, there is an alternative to the interpretation that the cell is a monocyte or monocyte hybrid (myelomonocyte). Recent observations that I made suggest that the cell in Fig. 4 of ref. 1 is a neutrophil myelocyte.

While studying a patient with systemic mastocytosis who progressed through the phase of an “unclassified” myeloproliferative disorder into acute myeloblastic leukemia, I noted a marked discrepancy between cytochemical reactivities and the morphology of the blood cells. The studies reported here were made during the year before he developed acute leukemia, at which time his peripheral leukocyte counts were elevated (20,000–35,000/\(\mu\)l, with 55%–60% neutrophils, 15%–16% monocytes). The bone marrow showed patchy dense fibrosis characteristic of mastocytosis, with the intervening marrow space markedly hypercellular. The neutrophilic series was predominant (65%–70% of nucleated cells), marrow monocytes were inconspicuous with Wright stain (< 4%), and myeloblasts were normal in number. Electron microscopy of the bone marrow showed neutrophilic predominance. Cytochemical studies with Sudan black B, peroxidase, and PAS stains\(^2\) were all consistent with a neutrophilic-type differentiation in 65%–70% of nucleated marrow cells. The AS-D-chloroacetate esterase stain\(^1\) showed 55%–60% of marrow nucleated cells with significant reactivity. Stains for NSE\(^3,4\) using either \(\alpha\)-naphthol acetate or butyrate as the substrate showed about 37% of the nucleated marrow cells to be strongly positive and an additional 22%–25% to be moderately positive. Less than 40% of cells showed negative or trace reactivity or contained the coarse focal positivity characteristic of some lymphocytes. All of the strongly and moderately positive cells showed marked sensitivity to fluoride inhibition. These results have been confirmed by repeated testing on both nonanticoagulated marrow smears (directs) and EDTA-anticoagulated smears of the marrow M-E layer (concentrates).

Because of the large population of NSE-positive cells, a pattern of reactivity in the neutrophilic series was obvious, with the greatest degree of positivity occurring in the promyelocyte-myelocyte stage and an abrupt decreased positivity in the very earliest and the later developmental stages.

With the experience gained from this case, I reviewed two other cases of hematopoietic dysplasia where we had noted a larger number of NSE-positive cells than the number expected from examination of the Wright stain, as well as another case of systematic mastocytosis associated with a myeloproliferative disorder. In each of these cases, it was possible to separate morphologically a group of cells similar in all respects to the neutrophil precursors previously identified and to trace NSE activity into smaller numbers of more mature forms.

Demonstration by Kass et al.\(^5\) of substantial quantities of neutrophilic \(\alpha\)-naphthol acetate and butyrate NSE activity that is at least partially fluoride sensitive supports these findings. Why this activity is not usually demonstrable, or why it is largely restricted to the neutrophil precursors, is unknown.

We perform the NSE stains in “benign” monocytes, but the potential hematologic malignancies are more intensively studied. Therefore the fact that strong neutrophil NSE positivity has been recognized only in hematopoietic dysplasias or myeloproliferative disorders may be misleading. It seems clear, however, that the recognition of this phenomenon—demonstration of NSE in early-middle maturation forms of neutrophils—may be an ancillary aid in the early identification of this group of patients.

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REFERENCES
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To the Editor:

In our cytochemical evaluation of acute leukemia specimens, we routinely use the AS-D-chloroacetate esterase (AS-D-CE) and a-naphthyl esterase (NSE) tests utilizing the "combined" method of Yam et al. Although technical problems occur more frequently with the combined method than when each esterase test is done separately, there are nonetheless obvious advantages. First, individual cells can be shown to have both NSE (monocyte) and AS-D-CE (neutrophil) reactivity as we described in the myelomonocytic leukemia. Second, there may be slightly increased efficiency in having two stains on one slide. Third, the "background" of NSE in myeloid cells is much fainter (very weak to absent), obviating the need for routine fluoride inhibition. Presumably Dr. Rydell tested separate esterase stains, which indicates that his observations may not be directly comparable to ours for technical reasons. Certainly we have noted more intense reactivity with NSE and AS-D-CE in individual stains; therefore terms such as "strongly" and "moderately" cannot be interpreted outside of the context of the technology employed.

An additional comment about the fluoride sensitivity of Dr. Rydell's "strongly or moderately" NSE-positive neutrophil precursors seems warranted. Evaluations of specimens from normal persons (?) and chronic granulocytic leukemia patients suggest that whatever minimal NSE is present in myeloid cells is at most weakly inhibited by fluoride. On the other hand, the NSE reactivity in a typical monocyte or the myelomonocyte in acute myelomonocytic leukemia is very sensitive to inhibition by fluoride. Dr. Rydell's landmark method of "strongly or moderately" NSE-positive neutrophil precursors seems warranted. Evaluations of specimens from normal persons (?) and chronic granulocytic leukemia patients suggest that whatever minimal NSE is present in myeloid cells is at most weakly inhibited by fluoride. On the other hand, the NSE reactivity in a typical monocyte or the myelomonocyte in acute myelomonocytic leukemia is very sensitive to inhibition by fluoride.

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RE Rydell