To the Editor:

In a recent report regarding the demonstration of Epstein-Barr viral DNA in acute large granular lymphocyte (natural killer) leukemic cells, a transmission electron micrograph (see Fig 1D of ref 1) is said to be an image of a peripheral blood large granular lymphocyte. The cell imaged is not a large granular lymphocyte. It is a section of a peripheral blood eosinophil that traverses one lobe of a typical granulocyte polylobed nucleus with a heavily condensed chromatin pattern. Peripheral blood eosinophils (Fig 1) are readily identifiable by their cytoplasmic organelles. These include variable numbers of bicompartmental, crystalline core-containing secondary granules, core-free primary granules, the storage site of Charcot-Leyden crystal protein, variable numbers of small granules, glycogen, mitochondria, and lipid bodies that are arachidonic acid-rich, prostaglandin endoperoxide synthase-containing (A.M. Dvorak and P.F. Weller, unpublished data, September 1992), membrane-free structures that are widely present in inflammatory cells. Eosinophils that are activated by variable means and in a number of diseases show quantitative changes in these cellular organelles. As in Fig 1D of ref 1, peripheral blood interstitial and cultured eosinophils can show marked reduction of their core-containing specific granules, increased numbers of primary and small granules, as well as increases in lipid bodies. Despite these changes, eosinophilic leukocytes can be distinguished from large granular lymphocytes by electron microscopy. In the case reported, neither the peripheral blood or bone marrow differential counts nor a total blood eosinophil count were presented. It is possible that a large granular lymphocyte proliferation existed in this patient but that an electron micrograph of an eosinophil was selected for publication. However, if, by electron microscopy, all the cells in question looked like the cell in Fig 1D, then the assumption that these are large granular lymphocytes must be re-evaluated.

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Fig 1. Mature human peripheral blood basophil from a patient with hypereosinophilic syndrome shows a bilobed nucleus (N), irregular, thick surface processes, granules, and mitochondria. Dark cytoplasmic particles are monoparticulate glycogen. Four large, round, osmiophilic lipid bodies (arrowheads) are present. Specific granules are elongated, membrane-bound structures with dense central crystals and lightly dense matrix (closed arrows). Primary granules do not have central crystals (open arrow). A few small granules are also present. Bar = 1.4 μm. Reprinted with permission.
We appreciate Dr Dvorak's interest in our report and are pleased to have the opportunity to comment on the suggestion that the electronmicrograph (Fig 1D) published in our report1 is of a cosinophil and not a large granular lymphocyte (LGL).

The electron microscopy studies were performed on peripheral blood leukocytes (white blood cell count: 28.3 x 10^3/L; differential: LGLs 44%, neutrophils 43%, lymphocytes 5%, monocytes 4%; eosinophils < 0.1%) at a time when no circulating eosinophils were present in the peripheral blood film. Furthermore, 61% of bone marrow cells were LGLs having irregular nuclei with cosinophil less than 0.1% of the marrow differential count. Immunophenotypic analysis of these cells, cytochemical (including peroxidase) staining and functional studies clearly identified the abnormal population of cells as being LGLs and not eosinophils. Electron microscope studies do not always readily distinguish the origin of leukemic cell types. The electron micrograph was presented for completeness of information and not to define the lineage of the abnormal cells in this patient. The conclusion that this was an LGL proliferation that contained Epstein-Barr viral DNA is correct.

The ultrastructural appearances of natural killer (NK) cells (LGLs) show electron dense membrane bound granules that contain acid phosphatase activity.2 Some investigators describe the presence of parallel tubular arrays (PTAs), but this is variable2 and may be absent from CD3-negative LGL leukemias.3 No PTAs were detected in our case. The ultrastructural appearances of the granules may vary considerably in normal LGL, and Grossi et al5 describe the smaller granules as having a fairly translucent matrix. Larger granules were reported to be more electron opaque and round heavily electron opaque inclusions were occasionally found within the granular matrix.3 Large abnormal granules may be a feature in neoplastic LGL proliferations and the ultrastructural features of the granules in several published cases6-10 appears similar to those noted in this case. Small very dense crystalloids have been associated with PTAs in leukemic LGLs.11

Re-examination of these cells confirms the presence of similar granules with central electron dense material (some elongated as in Fig 1D) within cells that have other lymphoid features. No cells with crystalline core containing secondary granules typical of eosinophils12 were observed. In other respects the morphology of the cell is consistent with an LGL leukemia with obvious mitochondria, lysosomes, and long granular endoplasmic reticulum. Glycogen is present as is described by Dvorak et al in mouse NK clones.13 Osmiophilic lipid bodies typical of eosinophils12 were not present. The ultrastructural appearances of the nucleus are entirely consistent with our description of the light microscopic findings of a relatively mature chromatin pattern. Vacuoles were also present in some LGLs at the light microscope level (see Fig 1A)

LGLs may share some features in common with myeloid cells.14 The ultrastructural similarities between the granules of mouse cytotoxic lymphocyte clones and basophils may be relevant12 and others have commented on the ultrastructural similarities between maturing LGLs and cells of the myelomonocytic lineage.5 The crystalloid free dense granules of eosinophils are not readily distinguished from LGL granules. Clearly more ultrastructural comparison of normal and neoplastic LGL with other cells will be informative and may identify features unique to these cell types.

The main aim of our report was to document the association of Epstein-Barr viral DNA with the abnormal cells of a leukemic LGL clone. Morphologic, surface marker, and functional analyses demonstrated without question that the leukemic cell was an LGL. Although it is always reasonable to debate the origins of a single cell at the EM level, the identity of the particular cell in question is not important to the overall conclusions presented.

REFERENCE


Eosinophils or large granular lymphocytes [letter; comment]

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