

### The value of autofluorescence as a diagnostic feature of acute promyelocytic leukemia

**Autofluorescence is an immunophenotypic characteristic of leukemic blasts in acute promyelocytic leukemia (APL). We examined the fluorescent intensity of isotype controls in 25 cases of APL and 25 controls with acute myeloid leukemia. The fluorescence of the FITC- and PE-conjugated controls was consistently higher in APL. Autofluorescence may therefore represent a helpful diagnostic marker in APL.**

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The diagnosis of acute promyelocytic leukaemia (APML) is currently made on a combination of morphological, immunophenotypic, cytogenetic and molecular genetic analyses.<sup>1</sup> The combination of low expression of HLA-DR, CD11a and CD18 represents a reliable surrogate antigen expression profile for PML/RAR $\alpha$ -positive APML.<sup>2</sup> Immunofluorescence with the anti-PML monoclonal antibody PG-M3 is another rapid and efficient method.<sup>3</sup> The integration of these assays facilitates the early instigation of appropriate therapy.<sup>4</sup>

Autofluorescence is the term used to describe the light emitted naturally by an unstained, illuminated cell. It can be estimated by determining the fluorescence of unstained cells and the background fluorescence of non-specific binding by including a negative control of a labeled, irrelevant monoclonal antibody.<sup>5</sup> While autofluorescence in APML is a recognized phenomenon in flow cytometry, little attention has been paid to its potential diagnostic utility. We therefore decided to quantify the degree of autofluorescence in 25 consecutive cases of APML and to compare the results with those of 25 cases of non-APML acute myeloid leukemia (AML). Twenty-five consecutive cases of APML presented to our center between 1993 and 2004. Morphologically, 23 cases had typical APML and two cases had the microgranular variant. Cytogenetic analysis detected the t(15;17) translocation in all 25 cases. Immunophenotyping studies were performed at diagnosis on erythrocyte-lysed whole bone marrow samples stained with monoclonal antibodies directly conjugated with fluorochromes. For staining, 100  $\mu$ L of RPMI/heparin-diluted bone marrow samples, containing  $2 \times 10^6$  nucleated cells, were placed in each tube and incubated with the appropriate combination of monoclonal antibodies. Isotype-matched mouse non-specific immunoglobulins were used as negative controls. Data acquisition was performed on a FACScan, or later a FACSCalibur, flow cytometer (Becton Dickinson) using the CELLQUEST PRO software program (Becton Dickinson). Of the 25 APML cases, 23 (92%) were CD34<sup>-</sup> and 23 (92%) were HLA-DR<sup>-</sup>. Four cases were positive (>30% in gated fraction) for either CD34 or HLA-DR. No cases were positive for both antigens. Twenty-four (96%) cases were CD13<sup>+</sup> and all 25 cases were positive for CD33 and myeloperoxidase. In early cases, apparent binding of all isotype-specific IgG and IgM controls and the corresponding test reagents at equivalent intensity suggested the possibility of Fc binding of the monoclonal reagents, a feature of acute myelomonocytic or monocytic leukemias.<sup>6</sup> This well-known phenomenon is caused

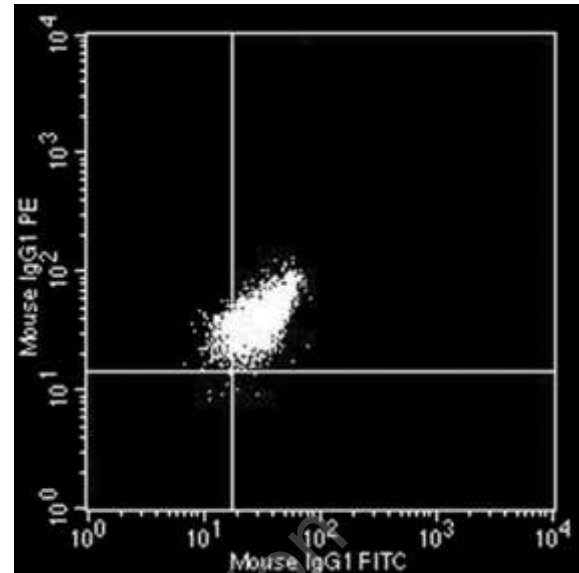
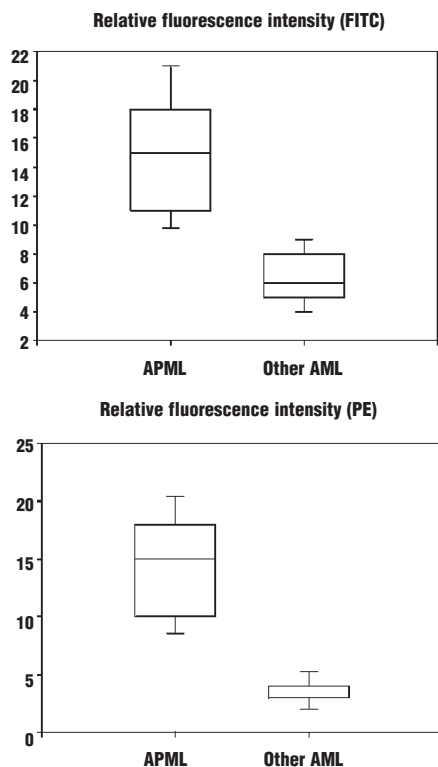


Figure 1. Autofluorescence detected using isotype controls.

by binding of mouse monoclonal antibodies to high-affinity Fc $\gamma$ RI receptors on the abnormal myeloid cells. This can usually be circumvented by prior incubation of the leukemic cells with aggregated human IgG. Such incubation, however, did not alter the pattern of staining. In the remaining cases, analysis of the cells in phosphate-buffered saline (PBS) alone without control or test monoclonal antibodies showed an identical fluorescence pattern. Control antibodies known as isotype controls are of the same immunoglobulin isotype or subclass as the staining antibodies used in the experiment but with a specificity unlikely to be found on human hematopoietic cells. Their role is to detect non-specific binding. Fluorescein isothiocyanate (FITC) – and phycoerythrin (PE) – conjugated isotype-matched negative controls were used in the immunophenotyping of all cases of AML in our laboratory during this 13-year period. In all 25 APML cases, the FITC-conjugated and PE-conjugated isotypic controls were shifted to the second log decade due to autofluorescence. An example is shown in Figure 1. The fluorescent intensity of non-APML AML isotypic controls was in the first log decade. The breakdown of this control group by FAB type was 5 cases of M1, 11 of M2, 7 of M4 and 2 of M5. In order to quantify the degree and specificity of autofluorescence, we compared the relative fluorescent intensity for both the FITC-conjugated and PE-conjugated isotype controls for the 25 APML cases and the 25 consecutive cases of non-APML AML (Figures 2A,2B). For both fluorochromes, autofluorescence was higher in APML cases than in any of the non-APML AML controls. No difference was detected between the cases of standard APML and the microgranular variants.

Background autofluorescence generally results from intracellular constituents such as flavins and pyridine nucleotides. Autofluorescence has been described in both eosinophils and neutrophils, due to the presence of granule-associated flavin adenine nucleotide and granule-associated NADPH, respectively.<sup>7</sup> It has also been reported as a feature of alveolar macrophages, which are derived from the bone marrow.<sup>8</sup> We are not aware, however, of studies on this phenomenon in APML.



**Figure 2.** Comparison of relative fluorescence intensity for FITC-conjugated (above) and PE-conjugated (below) isotype controls for the 25 APML cases and 25 consecutive cases of non-APML AML controls.

In summary, the presence of autofluorescence in leukemic blasts appears to be strongly suggestive of APML. This autofluorescence can be distinguished from the non-specific Fc binding of monocytic leukemic cells by observation of equivalent binding of all control anti-

bodies and an identical fluorescence pattern in both unstained cells and controls. Autofluorescence may, therefore, represent a helpful adjunctive diagnostic marker in APML.

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