A Neutrophil Membrane Marker Reveals Two Groups of Chronic Myelogenous Leukemia and Its Absence May Be a Marker of Disease Progression

By John I. Gallin, Robert J. Jacobson, Bruce E. Seligmann, Julia A. Metcalf, Jean H. McKay, Ronald A. Sacher, and Harry L. Malech

An IgG, monoclonal antibody, 31D8, that recognizes normal neutrophil (PMN) membranes, was used to study PMN from patients with chronic myelogenous leukemia (CML). Nineteen patients with Philadelphia chromosome positive CML were followed over a ten-month period and compared with 23 normals, six patients with leukemoid reactions, and eight patients with phagocytic cell defects. The percentage of PMN binding of 31D8 among normal subjects was variable about a normal distribution with an average of 95 ± 2% of cells binding 31D8. In contrast, there were two groups of CML patients: in 14 patients 88 ± 3% PMN bound 31D8 while in the remaining five patients only 6 ± 6% PMN bound 31D8. PMN 31D8 binding was normal in the control patient groups. Control antibodies 7C3 (binds to PMN precursors) and OKM1 (binds to the CR3 (IC3b) receptor) bound normally to CML neutrophils. Functionally, CML cells had normal chemotaxis to several stimuli and normal superoxide generation to phorbol myristate acetate. However, superoxide production in response to fmet-leu-phe was significantly less in 31D8 negative CML PMN than both 31D8 positive CML PMN and normal PMN which contained 85% 31D8 positive and 15% 31D8 negative PMN. Clinically, 2 of 14 CML patients with 31D8 positive PMN were in blast crisis (one extramedullary) at the time of study and the other 12 patients remained clinically stable in the chronic phase during the ten months of study. In contrast, one of five patients with 31D8 negative PMN was in blast crisis at the time of study and all four of the remaining patients progressed to either the accelerated phase or blast crisis. Three of these patients died of their disease eight to ten months after their initial study. Thus, failure of CML cells to bind 31D8 may be useful for predicting which patients are likely to progress to the accelerated phase or blast crisis.

HUMAN peripheral blood neutrophils have been shown to be heterogeneous by several parameters. We have previously described an IgG, monoclonal antibody that binds heterogeneously to human blood neutrophils. The antibody, 31D8, bound strongly to most, but not all, neutrophils with considerable variability among people. Neutrophil expression of 31D8 antigen appeared early in myeloid maturation, about the myelocyte stage. Additional studies indicated the 31D8 positive neutrophils performed better than 31D8 negative neutrophils for adherence to glass, and N-formylmethionylleucylphenylalanine (fmet-leu-phe)-stimulated chemotaxis and oxidative metabolism. In addition, the 31D8 positive cells exhibited a membrane potential depolarization when stimulated with FMLP while the 31D8 negative cells did not.

It is not known why there are two populations of neutrophils nor the origin of the heterogeneity in normal subjects. As part of our studies of the potential clinical importance of 31D8 expression, we initiated studies in patients with chronic myelogenous leukemia (CML). In this paper we present data that indicate that the majority of CML patients have normal neutrophil expression of 31D8 but in a minority (5 of 19 patients) nearly 100% of the neutrophils were 31D8 negative. Furthermore, the data indicate that failure of neutrophil expression of 31D8 antigen may be an early marker of progression of CML to the accelerated phase or blast crisis.

MATERIALS AND METHODS

Subjects. Nineteen patients with Philadelphia chromosome positive CML were studied over a 10-month period. Ten patients were females, three in blast crisis at the time of study, one extramedullary without blast cells detected in the blood or marrow. Nine patients were males. In addition, 23 normal subjects were studied in parallel, 12 females and 11 males. Control patients included 6 with leukemoid reactions with white blood counts ranging from 15,000 to 85,000 cells/mm³. Eight patients with phagocytic cell defects were also studied including four patients with chronic granulomatous disease, 1 patient with neutrophil myeloperoxidase deficiency, one patient with neutrophil-specific granule deficiency, and one patient with absent macrophage apolipoprotein E production.

Cell preparation and labeling with antibodies. Blood neutrophils were prepared by Hypaque-Ficoll and dextran sedimentation as described previously. Neutrophils were incubated with fluoresceinated mouse antihuman monoclonal antibody 31D8 for 30 minutes at 4°C and then washed × 3 with Hanks’ balanced salts solution as previously described. The cells were then fixed with 2% paraformaldehyde and saved for study in the fluorescent activated cell sorter. As recently described, 31D8 negative cells had slightly more fluorescence (less than 10%) than unstained cells whereas 31D8 positive cells exhibited at least 5 to 10 times greater fluorescence than unstained cells. In patients in blast crisis, identification of neutrophil precursors was done using parallel studies with a neutrophil-specific antibody we defined earlier called 7C3. In other control studies the commercially available mouse antihuman neutrophil antibody OKM1 was utilized. Wright’s stain was performed on cytospin preparations of all cells prepared for analysis in the fluorescent-activated cell sorter and differential counts were performed.

Neutrophil function. Neutrophil chemotaxis to buffer, fmet-
leu-phe (10⁻⁴ mol/L), endotoxin-activated serum (5% v/v), and sodium caseinate (5 mg/mL) was assayed by determining the average distance migrated by cells migrating into cellulose nitrate filters using a previously described technique. Superoxide generation in response to phorbol myristate acetate (PMA, 20 ng/mL) and fmet-leu-phe (10⁻⁴) was determined as the maximal rate of superoxide dismutase inhibitable cytochrome c reduction.

Statistical analysis. Except where indicated standard errors were used as an estimate of variance and Student's t test was used to determine the significance of differences.

RESULTS

31D8 binding of CML cells. The percentage of 31D8 positive cells was determined in CML and normal subjects (Fig 1). Normal neutrophils can exhibit two populations of cells, the majority of which are 31D8 positive and the rest 31D8 negative (Fig 1A). Two patterns were observed in neutrophils from CML patients. In one group the majority of cells bound 31D8 normally (Fig 1B) while in the other the majority did not bind 31D8 (Fig 1C). Figure 2 shows the distribution of the percentage of 31D8 positive neutrophils among normals and patients. The two groups of CML patients consist of subjects having greater than 60% of their neutrophils binding 31D8 (mean 88 ± 3%) and those with less than 30% of their cells binding 31D8 (mean 6 ± 6%, P < 0.001 v neutrophils from normal subjects or patients with 31D8 positive cells). The width of the gap between the two apparent distributions of CML data was larger than would be expected by chance alone (P < 0.02 for a normal distribution). Although some CML patients had low percentages of neutrophils binding 31D8, the intensity of 31D8 binding to 31D8 positive and negative cells was normal (data not shown). Previously we showed that 31D8 binding normally appears about the myelocyte stage. Differential counts on the cell preparations used for 31D8 binding studies indicated that the low percentage of 31D8 positive cells on some CML patients could not be accounted for by the overwhelming presence of immature neutrophils in these preparations. Four of five CML subjects with almost 100% 31D8 negative neutrophils had normal percentages of mature neutrophils, while the fifth patient, who was in blast crisis and had 0% 31D8 positive cells, had 17% peripheral blood neutrophil forms beyond the myelocyte state of maturation. 31D8 binding to neutrophils from patients with leukemoid reactions and neutrophil dysfunction was normal (93 ± 3% and 87 ± 5%, P > 0.05), and none of the patients with leukemoid reaction or with neutrophil dysfunction had a very low percentage of cells binding 31D8.

Binding of two other monoclonal antibodies, 7C3, an antibody that recognizes neutrophil lineages and OKM1, an antibody that binds to the α chain of the CR3 (iC3b) receptor, were also studied (Fig 3). Although there was some variability of 7C3 and OKM1 binding to CML neutrophils, unlike binding of 31D8, no CML patient neutrophils exhibited severe deficiency of 7C3 or OKM1 binding. Thus, the extreme heterogeneity of 31D8 binding to PMN from different CML subjects was specific for the 31D8 antibody with the three antibodies studied.

Neutrophil chemotaxis and superoxide generation. Eighteen studies of spontaneous nondirected locomotion to buffer and chemotaxis toward endotoxin-activated serum, fmet-leu-phe, or casein were performed on neutrophils from CML patients and concurrently run normals. With each stimulus tested the locomotive response to neutrophils from CML patients with greater than 60% 31D8 positive and less than 30% 31D8 negative neutrophils did not differ from normal cells (containing an average of 85% 31D8 positive and 15% 31D8 negative cells) or from each other (P > 0.05). Superoxide generation was normal in neutrophils from all CML patients when PMA was the stimulus, but when fmet-leu-phe was used as the stimulus a small yet significant impairment of superoxide generation in neutro-
phil patients indicates that these differences in the number of patients in blast crisis among the two patient groups were highly significant ($P = 0.002$, Fisher’s Exact Test).

**DISCUSSION**

The data in this paper indicate that, based on binding of the monoclonal antibody 31D8 to blood neutrophils, CML patients can be divided into two groups: those with neutrophils expressing normal 31D8 antigen and those with neutrophils lacking 31D8 antigen. The 31D8 negative neutrophils seen in some CML patients expressed normal amounts of another myeloid antigen, 7C3, showing specificity of the defect. The differences in 31D8 antigen expression in the two patient groups cannot be attributed to therapeutic regimens.

An analysis of the clinical course of the disease in two groups of CML patients was of interest. The 14 patients with 31D8 positive neutrophils included two patients in blast crisis, one of whom was in extramedullary blast crisis. All the
patients in the chronic phase remained clinically stable during the 10 months of study and none had 31D8 negative cells. In contrast, of the five patients with 31D8 negative neutrophils, one was in blast crisis at the time of study, three progressed to the accelerated phase, and one progressed to blast crisis within 10 months of initial study. Thus, all five CML patients in the 31D8 negative category either were in, or progressed to, the accelerated phase or blast crisis.

It is of interest that the locomotive responsiveness to numerous stimuli and superoxide generation in response to PMA of CML 31D8 positive and negative neutrophils were normal. The normal chemotactic responsiveness of CML 31D8 negative neutrophils was different from normal 31D8 negative neutrophils which have impaired chemotaxis compared with normal 31D8 positive cells. However, superoxide generation in response to fmet-leu-phe by cells from CML patients with 31D8 negative neutrophils was less than the response of cells from CML patients with 31D8 positive cells, similar to the results obtained between normal 31D8 positive and negative neutrophils.

The current data are compatible with the concept that heterogeneity of PMN 31D8 antigen expression results from different stem cell pools, yet the data by no means establish this as correct. However, it is intriguing that failure to express 31D8 antigen on neutrophils from patients with CML may be an early indicator of disease progression, although it is clear that some patients can progress clinically yet still express 31D8 antigen on their neutrophils. Although the number of patients in this study are too small to reach definitive conclusions, the possibility failure of neutrophil expression of 31D8 antigen is of prognostic use in most patients with CML is of sufficient clinical importance to justify intense clinical evaluation of 31D8 expression in CML, particularly with the improving results with bone marrow transplantation.

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REFERENCES

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