CLINICAL APPLICATION OF THE CYTOCHEMISTRY
IN HEMATOLOGY

TOSHIYUKI NAKATSUI AND HARUTO UCHINO

*Department of Internal Medicine, Research Institute for Nuclear Medicine
and Biology, Hiroshima University, Kasumi-cho, Hiroshima 734*

A good deal of efforts have been continued to differentiate the types of acute leukemia in terms of hematological cytochemistry. Almost all of the applicable cytochemistry were tried to stain the blood and bone marrow smears to find out some difference of materials or enzymes in the leukemic cells. Other cytochemical applications to clinical hematology were shown in Table 1.

Cytochemistry of leukemic cells

May-Giemsa stain is still one of the most important and basic staining method in clinical hematology. Recent popularization of phase-contrast microscopy made

TABLE 1. *Cytochemistry in clinical hematology*

Leukocytes	
Differential diagnosis of acute leukemia	Materials and Enzymes
Supportive diagnosis	Alkaline phosphatase
Screening test for CGD	NBT test
Erythrocytes	
Diagnosis of erythroleukemia	PAS reaction
Diagnosis of anemia	Sideroblast staining
	Supravital staining
Platelets	
Nothing peculiar	

TABLE 2. *Cytochemistry of the leukemic cells and neutrophils*

	AGL	CGL	MoL	ALL	CLL	ErL
Peroxidase	±	+	+	—	—	—
Sudan Black B	±	—~+	++	—	—	—
PAS	—~+	—~+	++	+~++	+	+
Alkaline Phosphatase	↑↑~↓	↓↓↓	↑	↑	→	→~↓
β-glucuronidase	D	D	DF	S	S	
Acid Pase	+	+	++	+	+	+
Esterase	+		++	++		
Aminopeptidase	—~+		++	—		
Dehydrogenase	+~++	+	++	++		
Phosphorylase	+	++	+++			

AGL, acute granulocytic leukemia; CGL, chronic granulocytic leukemia; MoL, monocytic leukemia; ALL, acute lymphocytic leukemia; CLL, chronic lymphocytic leukemia; ErL, erythroleukemia

it possible to differentiate the types of leukemic cells more accurately. But it is not very easy to differentiate the most immature undifferentiated leukemic "stem cells", even in electron microscopy. So we have to use as many kinds of cytochemistry as possible that is worth differentiating as shown in Table 2 (4, 6, 12, 13).

Peroxidase is well known to be stained in peroxisomes of myeloid cells and monocytes ultracytochemically. This is one of the most important and popular staining to differentiate lymphocytes and myeloid or monocytoid cells. Myeloid leukemic cells are sometimes peroxidase negative, but, if it is positive, it is sure that the cells are not lymphoid.

Sudan Black B is the staining for lipid substance and has similar findings with peroxidase.

PAS reaction stainings for glycogen and related mucopolysaccharide. It is characteristic in lymphoid blast cells in some cases (7, 10). Erythroleukemic erythroblasts have positive PAS reaction in the cytoplasm.

Lysosomal enzymes, such as β -glucuronidase, acid phosphatase, and non-specific esterase are high in monocytic leukemia.

Aminopeptidase, dehydrogenase, such as SDH, LDH, and G6PDH, and

FIG. 1. Neutrophil alkaline phosphatase in patient with chronic granulocytic leukemia whose neutrophil alkaline phosphatase is high. $\times 10,000$

phosphorylase are somewhat characteristic in each types of leukemia as shown in Table 2.

Alkaline phosphatase of the neutrophils is different from other cytochemistry, in the point that stained cells are not leukemic blast cells. This may be a secondary indicator of the disease, but, in patients with chronic granulocytic leukemia, the low activity seems to be pathognomonic.

These are applicable staining methods for blood and bone marrow smears, but practically, not so many kinds of cytochemistry are routinely used even in hematological clinical laboratories, except for a few special laboratories.

Ultracytochemistry of neutrophil alkaline phosphatase

Neutrophil alkaline phosphatase is one of the most actively studied cytochemistry in hematological field. We have reported ultracytochemical localization of neutrophil alkaline phosphatase (9). The enzyme activity is found in small vesicles, vacuoles, specific granules, and cell membrane of the neutrophils. In patients with chronic granulocytic leukemia, the enzyme positive structures are very few, but in special case of chronic granulocytic leukemia, whose alkaline phosphatase activity of the neutrophils is very high, the enzyme positive structures increased the numbers markedly (Fig. 1), as comparable with normals (Fig. 2).

FIG. 2. Neutrophil alkaline phosphatase of the normals. $\times 10,000$

FIG. 3. Neutrophil alkaline phosphatase of the normals after phagocytosis of staphylococcus. Enzyme activity is shown around the phagosomes. $\times 20,000$

FIG. 4. Neutrophil alkaline phosphatase in patient with chronic granulomatous disease, 15 minutes after incubation with staphylococcus. Different enzyme activity is shown in the phagosomes. $\times 20,000$

On phagocytosis, alkaline phosphatase activity is found to exist around the phagosomes, sometimes fusing with small enzyme-positive vesicles or granules (Fig. 3). As shown in Figure 4, some phagosomes have activity and others not. Some of the cells showed marked enzyme activity on the surface of the cell membrane in case of high neutrophil alkaline phosphatase (Fig. 5). On the other hand, in

FIG. 5. Neutrophil alkaline phosphatase in patients with chronic granulomatous disease whose neutrophil alkaline phosphatase is high, 15 minutes after incubation with staphylococcus. Eosinophils attached to neutrophils tightly and only neutrophil cell membrane has strong enzyme activity. $\times 6,000$

patients with chronic granulocytic leukemia, alkaline phosphatase do not increase after incubation with staphylococcus as shown in Fig. 6.

Figure 7 shows the schematic representation of alkaline phosphatase in human neutrophils. Cell membrane has enzyme activity when neutrophil alkaline phosphatase is very high, such as bacterial infection. Neutrophil cell membrane has a pinocytic or phagocytic function as other alkaline phosphatase positive absorptive cells, such as, microvilli of the brush border of duodenal absorptive cells (3), brush border of the tubular cells of kidneys (8), microvilli of the choroid epithelium (5) and pinocytic vesicles of intestinal capillary endothelium (2). The cell membrane might have functional differentiation to increase pinocytic or phagocytic function, when the enzyme activity is high. On the other hand, in cases of low neutrophil alkaline phosphatase, like chronic granulocytic leukemia, phagocytic function is just disturbed slightly (1, 11). The functional role of neutrophil alkaline phosphatase is not known clearly.

Clinical application of the cytochemistry in the hematological field is not largely in progress recently, but the elucidation of the cell function using cytochemistry is expected.

FIG. 6. Neutrophil alkaline phosphatase in patients with chronic granulocytic leukemia after incubation with staphylococcus. Enzyme activity is not shown. $\times 20,000$

FIG. 7. Schematic representation of alkaline phosphatase in human mature neutrophil.

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