

Neutrophil Surface Markers in Patients With Acute Leukemia

By Barbara G. Hofmeister, Carlos J. Carrera, and Sondra G. Barrett

The presence of complement and Fc receptors on neutrophils from patients with acute leukemia was investigated at different stages of the leukemic process. Both Fc receptors (Fc-H and Fc-R) and one complement receptor (C₃d) were normal when patients were studied at diagnosis, in relapse, and in remission. In contrast, the C₃b receptor was significantly reduced on the neutrophils at the time of diagnosis, but returned to normal levels when patients entered remission. Variable amounts of C₃b activity were

observed in patients in relapse, with approximately one-half of the patients showing decreased C₃b activity. To determine whether the reduction in C₃b receptors was specific for patients at diagnosis, three patients were studied at different times during their disease. Normal receptor levels were detected in these patients during remission, but the C₃b receptor was markedly reduced at diagnosis and at relapse.

THE INCIDENCE of severe and often fatal infections in patients with acute leukemia remains disturbingly high.¹ Although most of these infections can be attributed to an associated neutropenia, other infection-promoting factors must also be considered. Substantial evidence has accumulated to show functional defects in "normal" neutrophils from leukemic patients.²⁻⁵ Impaired phagocytic activity in such patients was first reported by Hirschenberg in 1939.⁶ Bactericidal defects in their neutrophils were subsequently noted.^{3,5,7} In addition, ultrastructural cytochemical evaluations have allowed the identification of abnormal neutrophils in acute myelogenous leukemia⁸ as well as in childhood acute lymphoblastic leukemia.⁹

For phagocytic killing to occur, receptors must be present and be able to bind to opsonized particles. Thus, a qualitative or quantitative defect in receptors may impair phagocytic function. This study was undertaken to determine if neutrophil membrane receptors were defective in patients with acute leukemia and whether the defect, if any, persists during the remission and relapse phases of the disease.

MATERIALS AND METHODS

Study Population

Fifty-two peripheral blood samples were obtained from 40 patients with acute leukemia. Clinical data on these patients are presented in Table 1. Bone marrow examination confirmed the diagnosis of acute leukemia. Patients were studied at diagnosis prior to initiation of chemotherapy.

Forty-nine control samples from healthy volunteers were tested in parallel. This investigation involving human subjects was carried out

according to the Declaration of Helsinki, and informed consent was obtained.

Isolation of Peripheral Blood Neutrophils

Peripheral blood was collected by venipuncture and anticoagulated with EDTA. Neutrophils were separated and purified according to the method of Barrett et al.¹⁰ The cells were adjusted to a concentration of 5×10^6 /ml in medium 199 containing 0.1% bovine serum albumin (199-BSA).

Preparation of Fc Receptor Reagents

Two types of IgG Fc receptors were detected, Fc-R and Fc-H.¹⁰ Trypsinized sheep erythrocytes were coated with subagglutinating amounts of rabbit 7s IgG antibody (Cordis Laboratories, Miami, Fla.) and resuspended in gelatin-veronal buffer (GVB) at 2×10^8 /ml to detect the Fc-R receptor. The reagent for Fc-H was prepared from human type O, Rh-positive red cells. These red cells were neuraminidase-treated, sensitized with human anti-Rh₀ (anti-D, Gamma Biologicals Inc., Houston, Tex.) and resuspended in GVB at 1.5×10^8 /ml. Prior to use, the Fc-H reagent was washed and resuspended in an equal volume of 199-BSA.

Detection of Fc Receptors

To detect the Fc-R receptor, 0.2 ml of isolated neutrophils were precooled and mixed with an equal volume of Fc-R reagent. This suspension was centrifuged at 200 g for 4 min and immediately placed on ice. The pellet was vigorously resuspended and cytocentrifuge slide preparations were made. After staining with Wright-Giemsa, these slides were examined microscopically. Cells with three or more attached RBC were considered positive rosettes. The Fc-H receptor was detected by mixing 0.2 ml of neutrophils with 0.2 ml of Fc-H reagent at room temperature for 30 min. The suspension was then centrifuged at 200 g for 10 min and slides were prepared and counted as above.

Preparation of C₃ Receptor Reagents

A reagent to detect C₃b receptors¹⁰ was prepared from human EDTA anticoagulated type O blood. The blood was washed in saline and coated with human cold agglutinin (IgM) and complement. After a 37°C incubation, the cold agglutinin was eluted off, leaving human red cells coated with C₃b. Trypsinization of the C₃b resulted in a reagent that was specific for C₃d receptors. Specificity of the reagents was tested by immune adherence. The C₃b reagent was defined as immune adherence positive while C₃d was negative.

Detection of Complement Receptors

The C₃b and C₃d receptors were detected by mixing equal volumes of neutrophils (0.2 ml) and the appropriate C₃ reagent and

From the Cancer Research Institute, University of California, San Francisco, Calif.

Supported in part by NIH Grant CA 520575 and American Cancer Society Grant PDT 85.

Submitted September 9, 1980; accepted October 4, 1980.

Address reprint requests to B. Hofmeister, 1282-M, Cancer Research Institute, University of California, San Francisco, Calif. 94143.

© 1981 by Grune & Stratton, Inc.
0006-4971/81/5702-0026\$01.00/0

Table 1. Clinical Diagnoses of Patients with Acute Leukemia

Stage of Disease	Clinical Diagnosis*	n
Diagnosis	ALL-Null	3
	ALL-Burkitt	1
	AML	3
	AMML	2
	AMoL	1
	SL	1
Relapse	ALL-Null	8
	ALL-Burkitt	2
	ALL-T	1
	AUL	1
	AMoL	1
	ALL AML	1
Remission	ALL-Null	18
	ALL-T	1
	AML	1

*ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; AMML, acute myelomonocytic leukemia; AMoL, acute monocytic leukemia; SL, smouldering leukemia; AUL, acute undifferentiated leukemia.

rotating the mixture for five minutes at 37°C. Cytocentrifuge slides were prepared and counted according to the above procedure.

Statistical Analysis

All data from patients were compared to those obtained from control subjects and analyzed for significance by the Wilcoxon two-sample rank sum test.

RESULTS

The mean percentage of positive rosettes for patients and controls is presented in Table 2. Receptors for Fc-R, Fc-H, and C₃d on neutrophils from patients did not differ significantly from those from control subjects, regardless of the type of leukemia or the stage of disease. In contrast, the C₃b receptor was significantly reduced in patients studied at diagnosis and in relapse but returned to normal during remission.

Ninety-three percent of the neutrophils from control subjects possessed C₃b receptors with only 1 of 49 normal subjects showing low activity. However, in patients studied at diagnosis, the mean percentage of neutrophils with C₃b receptors was 47%, with 10 of 11 patients showing low activity. Approximately one-half

of the patients at relapse had low C₃b receptors on their neutrophils with a mean of 61%, which was significantly lower than that of the controls. Values from patients studied during remission did not differ significantly from those of the control subjects. Eighty-nine percent of their neutrophils had C₃b receptors and only 3 of 24 patients studied showed low reactivity.

Results from the 3 patients studied at different stages of the leukemic process are presented in Fig. 1. In all cases, C₃b was reduced at diagnosis and relapse and was normal when the patient entered remission.

DISCUSSION

Increased susceptibility to bacterial and fungal infections in leukemic patients was formerly attributed to neutropenia alone. However, Bodey¹ reported that at any given granulocyte level, infection was present more often during relapse, which suggested that factors other than absolute numbers of PMN may play a role. Functional defects in neutrophils from patients with leukemia have been shown, but, because of the lack of standard methods for studying neutrophil function, conflicting results have been reported.

The results of this study provide additional evidence that in leukemic patients, qualitative defects may exist on their neutrophils that morphologically appear normal. We have found that C₃b receptors on neutrophils from these patients are significantly reduced at diagnosis and during relapse. However, during remission, the majority of patients showed normal levels of C₃b receptors on their neutrophils.

Various populations of leukemic neutrophils have been detected. Cell surface markers for C₃b have been found on most neutrophils from normal peripheral blood samples,¹⁰ but in leukemic patients a population of neutrophils lacks these markers. Electron microscopic evidence of abnormal populations of neutrophils in acute myelogenous leukemia (AML) has been reported.⁸ One population of PMN lacked azurophilic granules, another lacked specific granules, and the third population had both specific and azurophilic granules but no myeloperoxidase activity. Using

Table 2. PMN Fc and C₃ Receptors in Normal Subjects and Acute Leukemia Patients

	n	Percentage of Reactive Cells			
		Fc-R	Fc-H	C ₃ b	C ₃ d
Normal	49	94.5 ± 5.8*	3.5 ± 3.6	93.0 ± 3.8	13.6 ± 8.6
AL—diagnosis	11	94.5 ± 4.8	1.9 ± 1.4	47.1 ± 26.0†	8.3 ± 7.2
AL—remission	24	89.4 ± 12.8	5.4 ± 8.2	89.0 ± 8.1	20.5 ± 15.0
AL—relapse	17	93.0 ± 5.6	2.9 ± 3.4	61.0 ± 30.0‡	11.0 ± 8.7

*Mean ± SD.

†p < 0.0001.

‡p < 0.001.

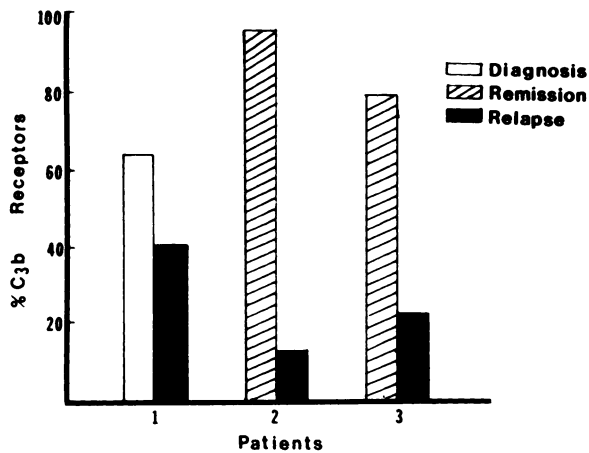


Fig. 1. Results of C_3b receptors on the neutrophils of three patients studied at different stages of their leukemia.

direct immunofluorescence, Rausch et al.¹¹ detected two populations of mature neutrophils in leukemic patients: those with surface antibody to myeloperoxidase, cathespin G, elastase, lysozyme, and lactoferrin, and those lacking one or more of the cytochemical markers.

Although we assume that neutrophils that lack C_3b receptors may be derived from a leukemic clone in myeloid leukemia, other explanations must be given for this because of the similar finding in lymphoid leukemia. Abnormal receptors could not be a result of chemotherapeutic effects in our patients studied at

diagnosis, since all tests were done before treatment. However, when studying patient neutrophils at relapse, the type and duration of therapy must be further assessed. We can assume that maintenance therapy did not affect the receptor tests of our patients, since most patients with ALL in remission and on maintenance therapy had normal receptors.

Other factors must be considered to account for low neutrophil C_3b activity. Whether toxic substances produced by leukemic blasts might alter receptor activity is open to question. Broxmeyer et al.¹² found that cells in the bone marrow of patients suffering from both myeloid and lymphoid leukemia produce a leukocyte inhibitory factor. Such a factor might disturb the membrane of normal neutrophils and alter C_3b receptor expression. Or, perhaps, these neutrophils are less mature normal cells that have been prematurely released from the bone marrow, and therefore have reduced C_3b . Another explanation may be due to asynchronous maturation in leukemia.¹³ Normal appearing neutrophils may actually be leukemic cells with immature membranes, thus the low receptor activity.

In addition to identifying abnormal neutrophils with perhaps defective function, C_3b reactivity may also be diagnostically valuable. Reduced C_3b receptors on blood neutrophils of leukemic patients may be useful early indicators of relapse detectable before significant numbers of circulating or bone marrow leukemic blasts could be detected.

REFERENCES

1. Bodey GP, Buckley M, Sathe YS, Freireich EJ: Quantitative relationships between circulating leukocytes and infection in patients with acute leukemia. *Ann Intern Med* 64:328, 1966
2. Coiffier B, Frobert Y, Revol L: Polymorphonuclear function in acute myeloblastic leukemia. *Biomedicine* 27:94, 1977
3. Humbert JR, Hutter JJ, Thoren CH, Dearnley PA: Decreased neutrophil bactericidal activity in acute leukemia of childhood. *Cancer* 37:2194, 1976
4. Child JA, Martin S, Cawley JC, Ghoneim ATM: Defective microbicidal function of neutrophils in haematological malignancies and lymphomas: Corrections by levamisole in vitro. *Biomedicine* 29:159, 1978
5. Wilkinson PM, Sumner C, Delamore IW, Geary CG, Milner GR: Granulocyte function in myeloblastic leukemia. *Br J Cancer* 32:574, 1975
6. Hirschenberg N: Phagocytic activity in leukemia. *Am J Med Sci* 197:706, 1939
7. Baehner RL, Neiburger RG, Johnson DE, Murrmann SM: Transient bactericidal defect of peripheral blood phagocytes from children with acute lymphoblastic leukemia receiving craniospinal irradiation. *N Engl J Med* 289:209, 1973
8. Bainton DF: Abnormal neutrophils in acute myelogenous leukemia: Identification of subpopulations based on analysis of azurophil and specific granules. *Blood Cells* 1:191, 1975
9. Pituch A: Peripheral blood neutrophils in children in remission of acute lymphoblastic leukemia. *Cytochemical investigations. Acta Med Pol* 18:69, 1977
10. Barrett S, Garratty E, Garratty G: Cell surface heterogeneity of human blood neutrophils and monocytes. *Br J Haematol* 43:575, 1979
11. Rausch PG, Pryzwansky KB, Spitznagel JK, Herlov JC: Immunocytochemical identification of abnormal polymorphonuclear neutrophils in patients with leukemia. *Blood Cells* 4:369, 1978
12. Broxmeyer HE, Ralph P, Margolis VB, Nakoinz I, Meyers P, Kapoor N, Moore MAS: Characteristics of bone marrow and blood cells in human leukemia that produce leukemia inhibitory activity (LIA). *Leuk Res* 3:193, 1979
13. Bessis M: *Blood smears reinterpreted*. Paris, Springer, 1977, p 208