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The Role of the Mature Neutrophil in Bacterial Infections in Acute Leukemia

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THE natural history of acute leukemia is characterized by the development of frequent and severe bacterial infections. This suggests that susceptibility to infection may be related to impaired cellular or humoral mechanisms of host resistance, a consequence of the marked derangement of the hematopoietic system in this disease. Although measurements have previously been made of certain factors of cellular defense against infection in patients with acute leukemia, the reports are conflicting. While Hirschberg[1] found that phagocytosis was impaired in acute leukemia, Braude et al.,[2] Strumia and Boerner,[3] and Jersild[4] observed that the phagocytic activity of the mature neutrophil in leukemic individuals was normal. Braude et al. stated that in acute leukemia the susceptibility of certain patients to bacterial infection was a function of a marked decrease in number of mature neutrophils, rather than a decrease in phagocytic function of the individual cell. However, other authors[1, 5, 6] have recorded the development of severe bacterial infections when the absolute mature neutrophil count was high (due to high total white blood cell count). Our own clinical observations have confirmed this; conversely, we have observed that some leukemic patients, with relatively low mature neutrophil counts, do not appear to be extraordinarily susceptible to infection during various phases of their illness.

Recent reports have directed attention to the relationship of infections and remission in acute leukemia. The onset of infection has been discussed in relation to total leukocyte and granulocyte counts. Diamond and Luhby[7] reported that remission was characteristically preceded by a marked peripheral leukopenia and hypoplasia of the marrow. Severe infection antedated the leukopenic-hypoplastic phase in the majority of the "complete" and partial remissions. Bassen and Kohn[8] questioned whether the infections noted by Diamond and Luhby were the result and not the cause of the leukopenia. Bierman et al.[5] observed that in children with acute leukemia, infection developed with a high or low leukocyte count, and with or without a marked granulocytopenia.

Detailed clinical observations on the nature of infections in patients with acute leukemia were made during the past year at the Clinical Center, National Cancer Institute, and have been reported in another communication.[9] As part of this study, observations were made of the relationship of the number of circulating mature neutrophils to the onset of bacterial infection. Phagocytosis of Brucella organisms by mature neutrophils in vitro was studied. The results of these investigations are given in this paper.

Methods and Materials

1. Clinical observations were made on thirty-six patients with active acute leukemia between March 15, 1955 and March 1, 1956. The period of observation for each patient ranged from 2 to 196 days, the median being 88 days. The diagnosis of acute leukemia was made in

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each case by examination of the bone marrow aspiration with supravital and fixed preparations employing Janus green and Giemsa stains respectively. The bone marrow criteria used to classify the activity of the disease were those accepted by the Clinical Studies Panel of the Cancer Chemotherapy National Service Center.[10] Hemoglobin, platelet count, leukocyte count and differential were performed routinely three times weekly. The total white blood cell count was determined after filling both chambers of a standard hemacytometer from one pipet and counting the cells in the four large squares of each chamber. A smear of the peripheral blood was prepared with Giemsa stain, and 200 cells were counted to obtain the differential. (When the total leukocyte count was 200/Cu. mm or less, only 50 cells were counted.) The diagnostic methods employed to determine bacterial infection, and the infections encountered are reported in a separate communication.[9] Drugs used in the treatment of acute leukemia during this period were 6-mercaptopurine, 4-amino-N^{10}-methyl pteroylglutamic acid (methotrexate), corticotropin, and cortisone and other structurally related steroids.

Phagocytic studies were performed in ten patients who had not received prior steroids, and were repeated when infection or hematologic remission occurred. When retests were performed, there was also no prior administration of steroids.

2. The phagocytic system employed consisted of the patient's whole citrated blood (0.8% final concentration of sodium citrate) incubated in the presence of live, virulent Brucella organisms which had been previously sensitized by exposure to graded amounts of brucellosis-immune serum.

a) *Preparation of Brucella culture for use in phagocytic study.* The Brucella culture selected for use was a smooth, highly virulent strain of *Brucella abortus* recently isolated from a human infection. The guinea pig infectivity was determined just before its use in this study. The ID_{50} was less than ten viable organisms (actual determination was two). For use in the phagocytic studies, the forty-eight hour growth on liver agar at 37 C. was suspended in physiological saline and adjusted with the aid of a Fisher electrophotometer to an estimated 25×10^9 organisms per ml. During the intervals between tests, the culture was maintained on liver agar slants stored at 5 C.

b) *Test procedure.* The test procedure was carried out in two parts. In part I, the routine Huddleson technic[11] for measuring the ability of leukocytes to phagocytize Brucella was employed. Preliminary tests indicated that the concentration of leukocytes did not affect phagocytosis. Two controls were run in parallel with the patient test: whole citrated blood of a known brucellosis-immune subject and blood from a normal brucellosis-negative individual. (This test was made to assure that the bacterial culture was in the proper antigenic state, i.e., the Brucella would be readily phagocytized by leukocytes of the immune control and would not be phagocytized by normal leukocytes in the absence of immune serum. It also indicated whether the leukemic patient possessed *Brucella opsonins* due to previous exposure.) When this test indicated that both the leukemic blood and that of the normal control showed no phagocytosis of the Brucella, while that of the immune control did, the second part of the test was undertaken.

In part II, the test procedure was a modification of the method described by Jersild[12] for titration of Brucella opsonins. A stock supply of immune serum was obtained by bleeding a healthy research technician who had been continuously exposed to Brucella organisms for the past ten years and whose blood, when tested periodically over the past four years, showed marked phagocytic ability for Brucella. The serum specimen was stored in the frozen state one month prior to its use. For use in the present study, it was thawed and maintained in a sterile screw cap vial; storage during the intervals between tests was at 5 C. The serum contained no preservative. It had a Brucella agglutinin titer of 1:640.

Parallel tenfold dilutions were made of the immune serum, using as diluent in one series, the serum from leukemic blood and in the other series, serum provided by the normal control blood. By means of a serologic pipet (0.2 ml. $\times$ 0.001), 0.02 ml. volumes of immune serum, undiluted, and after dilution, were transferred to 12 $\times$ 100 mm. glass tubes. To each tube was added 0.1 ml. of a standard suspension of Brucella and the tubes were placed in 37 C. incubator for thirty minutes. To the appropriate series was then added 0.1 ml. of the whole citrated leukemic blood; the parallel series received a similar quantity of normal whole citrated blood. The final concentration of immune serum in the tubes was 1:10, 1:100,

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and 1:1000. In some instances, a dilution of 1:10,000 was tested. The tubes were re-incubated for an additional thirty minutes with periodic mixing of the tube contents at least three times. Immediately following the incubation period blood smears were prepared using a separate capillary pipet to remove samples from each tube. Prompt drying of the films was facilitated by the use of a flow of warm air. Fixing and staining of the smears was carried out by the procedure developed by McCullough and Dick[13] for use in the Brucella opsonocytophagic test.

c) *Measurement of degree of phagocytosis.* The stained smears were examined microscopically and the number of Brucella ingested per leukocyte was counted for a total of 25 leukocytes. It was observed that in a given phagocytic preparation, the degree of phagocytosis of individual mature neutrophils was relatively constant. Based on the method by Huddleson, total phagocytic activity of the preparation was thus graded as follows: leukocytes which had ingested forty or more Brucella were graded as showing 3+ phagocytosis; those which contained 20-40 organisms as showing 2+ phagocytosis; those which contained 1-20 Brucella as showing 1+ phagocytosis; leukocytes which contained no Brucella were graded as zero. Only mature neutrophils were included in the examination. Mature neutrophils were defined as band and segmented forms. Immature neutrophils were defined as metamyelocytes, myelocytes, progranulocytes and myeloblasts.

Results

Table 1 records the age, the sex, and the hematologic diagnoses of the ten patients in whom the phagocytic studies were performed. The white blood cell count and differential on the day of the test are given. It was possible to repeat the phagocytic tests during severe infection in two patients with active hemato-

TABLE 1.—*Clinical Data Pertaining to Phagocytic Studies*

Pt. No.	Age	Sex	Type leukemia	Status of leukemia	Clinical infection	Bacterium	WBC × 10³	Differential*									
								SN	BdN	MM	M	MBl	L	A.L.	M_o	E_o	B_a
1	3	M	Lymphocytic	Active	None		3.5	10					89		1		
				Remission	None		4.5	38					57		4		1
2	8	M	Lymphocytic	Active	None		3.1	3					67	30			
				Remission	None												
3	6	F	Lymphocytic	Remission	None		2.4	63					34		0	3	
				Remission	Cellulitis	Ps. aeruginosa	1.3	16					82		0	2	
4	7	F	Lymphocytic	Active	None		50.0	7						93			
5	22	M	Lymphocytic	Active	None		4.8	46	9	6	6		25	7	1		
6	34	F	Lymphocytic	Active	None		11.1	15		2			36	38	7	2	
7	49	F	Lymphocytic	Active	Pyelonephritis	E. coli	15.2	2	1	1			15	81			
				Active	Pharyngitis	Ps. aeruginosa	0.68	37					63				
				Remission	None		9.4	67					32				
8	47	M	Granulocytic	Active	None		127.0	4			71	12	13				
				Active	Cellulitis	Ps. aeruginosa	1.0	16			28	48	8				
				Partial Remission	None		1.4	29					64			7	
9	47	M	Granulocytic	Active	None		3.9	52			43				1	3	1
10	28	F	Granulocytic	Active	None		2.6	26			2	41	30			1	

* *Key:* SN—segmented neutrophils; BdN—banded neutrophils; MM—metamyelocyte; M—myelocyte; MBl—myeloblast; L—normal lymphocyte; AL—abnormal immature lymphocyte ("lymphoblast"); M_o—monocyte; E_o—Eosinophil; B_a—Basophil.

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logic disease (Nos. 7, 8), in one patient with severe infection during complete hematologic remission (No. 3), and in four patients during partial or complete remission without infection (Nos. 1, 2, 7, 8). Three patients (Nos. 5, 9, 10) had demonstrable phagocytosis prior to the addition of immune serum (sera from these patients did contain brucella agglutinins). In patients 9 and 10, phagocytosis was 3+, and in patient 5, 1 to 2+. This compared to 3+ phagocytosis in the immune control and zero phagocytosis in the non-immune control.

In the remaining seven patients (Nos. 1, 2, 3, 4, 6, 7, 8) and in the non-immune controls, no phagocytosis occurred when the Brucella were incubated in the absence of immune serum. With the addition of 0.02 ml. of immune serum, the mature neutrophils from leukemic patients and normal controls showed 3+ phagocytosis. With 0.002 ml. of immune serum, phagocytosis in leukemic and control blood was 3+ in five patients and their controls, and 1+ in two patients and controls. With 0.0002 ml. of immune serum, phagocytosis by mature neutrophils of leukemic and control blood was 1+ to 0.

The total amount of antimetabolite drug received to the day of each phagocytic test was calculated for every patient. Varying amounts of 6-mercaptopurine and methotrexate had been administered prior to each test. Amounts ranged from 0 to 61 Gm. of 6-mercaptopurine and 0 to 1 Gm. of methotrexate.

Relationship of Mature Neutrophil Count to Infection in Acute Leukemia.

The opsonocytophagic system as employed in our series of tests corroborated previous impressions that phagocytosis by morphologically normal mature neutrophils was not impaired in acute leukemia. It seemed reasonable to determine next the relation of bacterial infections in patients with acute leukemia to the mature neutrophil counts in the peripheral blood.

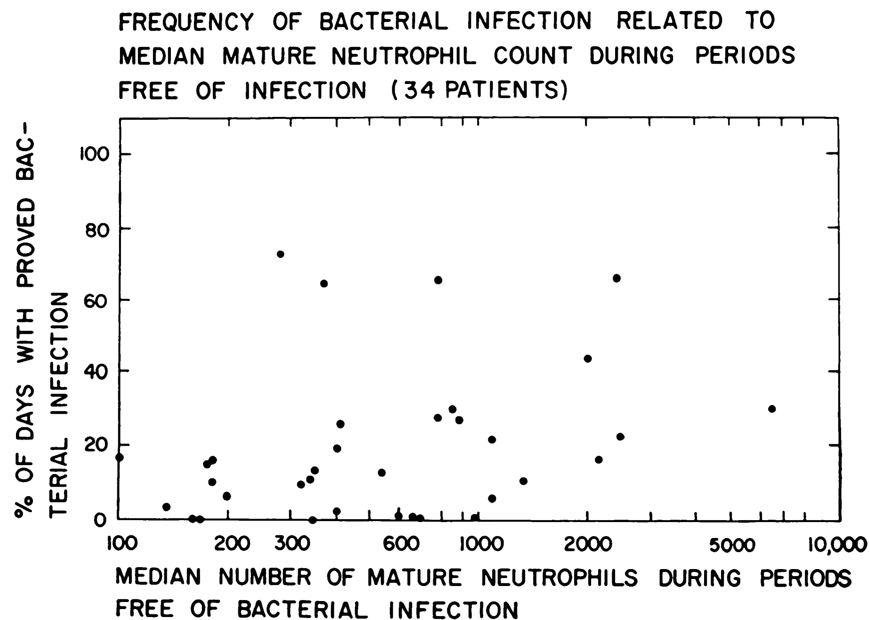

FIG. 1

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As Best et al.[14] have observed, computation of mean counts is of little value in leukemia because of their skewed distribution. Therefore, median mature neutrophil (MN) counts and median leukocyte counts minus the mature neutrophil counts (L − MN) were computed for all 36 patients for the period with and without proved bacterial infection. (Two patients had no infection-free periods.)

The median MN count (as well as the average per cent of mature neutrophils) during infection-free periods could not be correlated with the frequency of infection in the individual patient (fig. 1). This indicated that in general patients with lower MN counts in periods free of infection did not subsequently develop more infections than patients with higher MN counts. The change in the MN count immediately prior to the development of a proved bacterial infection was then examined.

Serial hematologic studies were available prior to, during, and following a proved bacterial infection in eighteen patients. In seventeen of these patients, infection was preceded by a drop in the MN count immediately prior to the onset of infection. The probability that this could occur due to chance alone in seventeen of eighteen patients (assuming that a 50–50 division is expected) is less than .0002.[15] Figure 2 depicts for the eighteen patients the ratio of the MN count prior to the development of infection to the median NM count in the period free of infection. The median decrease for the eighteen patients was fifty-four per cent. Although bacterial infection was thus *preceded* by decrease in the mature neutrophil count, it is to be emphasized that infection did not invariably develop following *every* decrease.

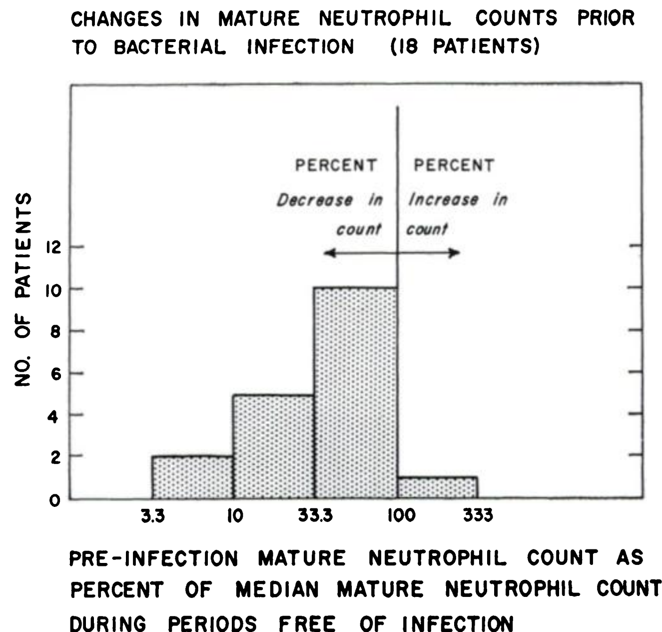

FIG. 2

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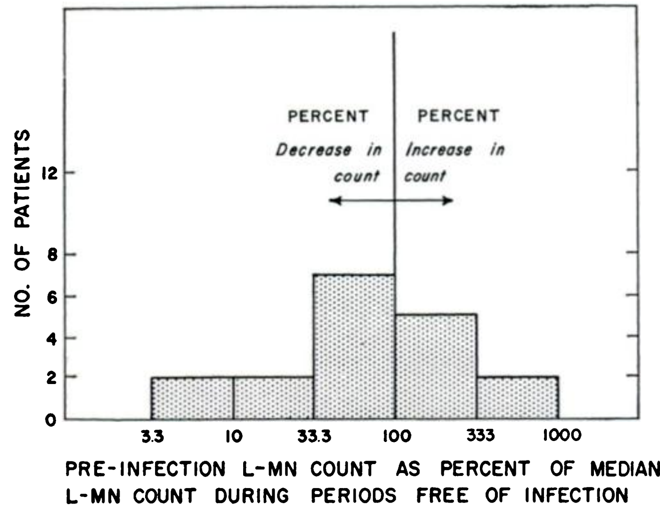

FIG. 3

The next consideration was whether this relationship was also true for all leukocytes other than mature neutrophils. Similar calculations were made. Prior to the onset of proved infection, eleven patients had falls in the L-MN count and seven had rises. The probability that this division could occur due to chance alone is 0.24.[15] The median decrease in L-MN counts for the eighteen patients was six per cent (fig. 3).

DISCUSSION

This experience suggests that phagocytosis of Brucella organisms by mature neutrophils in patients with active acute leukemia is not impaired. There was no evidence that phagocytosis was altered by antimetabolite therapy. The occurrence of infection was not associated with changes in phagocytic activity as measured by this test either during active hematologic disease or during remission. Other evidence that the phagocytic activity of mature neutrophils in acute leukemia is not impaired is based upon the results obtained when staphylococci and pneumococci were employed in the test system.[2, 3]

While only phagocytosis by mature neutrophils was quantified, we observed that immature cells of the granulocytic and lymphocytic series displayed no significant phagocytic activity. This is in agreement with previously published results.[2-4] Thus, the development of bacterial infection appeared to be preceded by, but did not necessarily follow, dynamic decreases of the order of fifty per cent or more in the mature neutrophil counts and not by changes in other leukocytes. (Neutropenia of this degree could have developed during the course of acute leukemia as a consequence of either the disease per se or antimetabolite therapy.)

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The importance of change in the MN counts is further emphasized by the fact that the median MN count in periods free of infection could not be correlated with the frequency of bacterial infections in a given patient. This suggests that in acute leukemia, an individual MN count, high or low, in a period free of infection is not necessarily of prognostic value for predicting susceptibility to future infection.

The absolute MN count when infection eventually developed varied from patient to patient. Not infrequently, infection occurred at significantly different MN levels within the same patient. Although bacterial infection was preceded by decreases in the mature neutrophil count, it did not invariably follow such changes. Thus, probably more than one factor accounts for the frequency of bacterial infections in patients with acute leukemia.[9]

Summary

In acute leukemia, phagocytosis of Brucella organisms by mature neutrophils was not impaired. Phagocytosis of these organisms was not altered by antimetabolite therapy or during periods of bacterial infection. The absolute mature neutrophil count when infection developed was found to vary from patient to patient. The onset of bacterial infection in patients with acute leukemia was preceded by dynamic decreases in the number of mature neutrophils in the peripheral blood. Seventeen of eighteen patients had lower mature neutrophil counts immediately prior to the onset of infection as compared to the period free of infection. The median decrease was 54 per cent. As infection did not always follow decreases in mature neutrophils it is suggested that other factors also play a role in the development of bacterial infections in patients with acute leukemia.

Summario in Interlingua

In leucemia acute, le phagocytose de brucellas per neutrophilos matur se monstrava intacte. Le phagocytose de iste organismos non esseva alterate per therapia a antimetabolitos o durante periodos de infection bacterial. Le numeration absolute de neutrophilos matur in stato de infection variava ab un patiente al altere. Le declaration de infection bacterial in patientes con leucemia acute esseva precedite per dynamic reductiones del numero de neutrophilos matur in le sanguine peripheric. Dece-septe ex dece-octo patientes habeva reducite numerationes de neutrophilos matur immediatemente ante le declaration de infection in comparation con le periodo libere de infection. Le reduction median esseva 54 pro cento. Viste que infection non sequeva le reduction del neutrophilos matur in omne casos, il debe esser concludite que altere factores es etiam implicate in le disveloppamento de infectiones bacterial in patientes con leucemia acute.

References

[1] Hirschberg, N.: Phagocytic activity in leukemia, Am. J. M. Sc. *197:* 706, 1939.

[2] Braude, A., Feltes, J., and Brooks, M.: Differences between the activities of mature granulocytes in leukemic and normal blood. J. Clin. Invest. *33:* 1036, 1954.

[3] Strumia, M. and Boerner, F.: Phagocytic activity of circulating cells in the various types of leukemias. Am. J. Pathol. *13:* 335, 1937.

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[4] JERSILD, M.: Phagocytic activities of various types of leukocytes. Acta med. Scandinav. (Supplement) *213:* 238, 1948.

[5] BIERMAN, H., CRILE, D., DOD, K., KELLY, K., PETRAKIS, N., WHITE, L., AND SHIMKIN, M.: Remissions in leukemia of childhood following acute infectious disease. Cancer *6:* 591, 1953.

[6] SOUTHAM, C., CRAVER, L., DARGEON, H., AND BURCHENAL, J.: A study of the natural history of acute leukemia with special reference to the duration of the disease and the occurrence of remissions. Cancer *4:* 39, 1951.

[7] DIAMOND, L. AND LUHBY, A.: Pattern of "spontaneous" remission in leukemia of childhood observed in 26 of 300 cases. (Abstract) Am. J. Med. *10:* 238, 1951.

[8] BASSEN, F. AND KOHN, J.: Multiple spontaneous remissions in a child with acute leukemia. Blood *7:* 37, 1952.

[9] SILVER, R. UTZ, J., FREI, E., ZUBROD, C., AND MCCULLOUGH, N.: Infections, fever, and host resistance in acute leukemia. Proceedings, VI International Hematology Congress, 1956. In press.

[10] Clinical Studies Panel, Cancer Chemotherapy National Service Center, Criteria for the evaluation of response to treatment in acute leukemia. (Letter to the editor) Blood *9:* 676, 1956.

[11] HUDDLESON, I., Brucellosis in man and animals. New York, Commonwealth Fund, 1943.

[12] JERSILD, M.: A cytophagic reaction employed in the diagnosis of brucella infection. J. Infect. Dis. *68:* 16, 1941.

[13] MCCULLOUGH, N. AND DICK, L.: A staining procedure for use in the brucella opsonocytophagic test. Stain Technology *17:* 153, 1942.

[14] BEST, W., LIMARZI, L., AND PONCER, H.: White blood cell counts in the leucemias. (Abstract) J. Lab. & Clin. Med. *38:* 789, 1951.

[15] Computation Laboratory, Harvard University, Tables of the cumulative binomial probability distribution. Cambridge, Mass., Harvard University Press, 1955

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