Defective Mononuclear Phagocyte Function in Patients with Myelomonocytic Leukemia and in Some Patients with Lymphoma

MARTIN J. CLINE

From the Cancer Research Institute, University of California School of Medicine, San Francisco, California 94122

ABSTRACT Mononuclear phagocytes from 8 of 10 patients with myelomonocytic leukemia and 2 of 9 patients with lymphoma phagocytized several species of bacteria but did not inhibit intracellular bacterial replication normally. Intracellular organisms were protected from the lethal effects of antibiotics in the medium. This defect of microbicidal function of malignant monocytes may explain in part the frequency of infection and the mechanism of antibiotic-resistant infection in some patients with malignant myeloproliferative and lymphoproliferative diseases.

INTRODUCTION

Malignant mononuclear leukocytes from human, as well as murine, myelomonocytic leukemia are proliferating cells, as evidenced by nuclear incorporation of tritiated thymidine and mitotic activity. These cells demonstrate some of the characteristics of mature, normal monocytes and macrophages, including adherence to a charged surface, phagocytosis, and the presence of surface receptors for IgG immunoglobulins (1–3). We recently observed that mononuclear phagocytes from most patients with acute myelomonocytic leukemia and from some patients with lymphoma were defective in microbicidal assays. These cells were capable of ingesting bacteria but were defective in their ability to inhibit the replication of ingested organisms. The intracellular bacteria were protected from some extracellular antibiotics.

METHODS

Patients studied. 15 normal subjects, 15 patients with nonhematologic malignancy, 10 patients with acute myelomonocytic leukemia, one patient with chronic monocytic leukemia, and nine patients with non-Hodgkin's lymphoma were studied (Table I). All patients were free of infection at the time of study. The cases with myelomonocytic leukemia comprised a spectrum that varied from predominantly myeloblastic disease (90% myeloblasts and promyelocytes) to predominantly monoblastic proliferation (58% monoblasts). Six of these patients had received extensive chemotherapy prior to study; four patients had never been treated. The patient with "chronic" monocytic leukemia had a chronic disorder characterized by anemia, thrombocytopenia, hepatosplenomegaly, and 10,000–12,000/mm^3 mononuclear cells of variable morphologic maturity in her circulation.

Bacterial growth. Bacteria grown overnight in trypticase soy broth were washed by centrifugation with saline or Zobell's solution (*Listeria monocytogenes*, ref. 4) and suspended at a concentration of approximately 2×10^8 colonies/ml. $\frac{1}{10}$ ml of the suspension was inoculated into 0.9 ml of McCoy's medium containing 30% normal human AB serum (ABM) and incubated at 37°C for 60 min when greater than 50% of the bacteria were in DNA synthesis. These log phase organisms were used for microbicidal assay.

Microbicidal assays. Leukocytes were isolated from heparinized peripheral blood (5) and tested in one or both of two microbicidal assay systems: (*a*) a standard viable colony count technique (5) based on the method of Hirsch and Strauss (6); (*b*) an assay system based on the incorporation of [^{3}H]thymidine by intracellular bacteria replicating within morphologically identifiable leukocytes (7, 8). In the latter system, 1 drop of heparinized blood was mixed with 1 drop of ABM on an eight-chambered sterile slide (Lab-Tek Products, Westmont, Ill.) and allowed to stand without agitation at 37°C in a humidified incubator. After 60 min, nonadherent cells were gently washed off with warm Hanks' solution, and 1 drop of ABM containing log phase bacteria was added. Incubation for 30 min at 37°C was allowed for phagocytosis before nonphagocytized bacteria were washed off with warm Hanks', and 1 drop of ABM containing 1 μCi/ml [^{3}H]thymidine (more than 20 Ci/mM) was added to label intracellular bacteria. After 30 min, unbound thymidine was removed by washing; the cover slips were fixed in methanol and mounted for dipping into Kodak NTB autoradiography emulsion. After exposure for 5 days the slides were developed and stained with Giemsa.

Received for publication 26 December 1972 and in revised form 2 May 1973.

TABLE I
Clinical Material

Disease category	No. studied	Age	Leukocytes/mm³	Mononuclear cells	No. receiving chemotherapy
		yr		%	
Normal	15	6–67	5,000–8,000	2–7	None
Cancer	15	18–70	4,200–12,000	2–10	11
Acute myelomonocytic leukemia	10	22–69	2,200–33,000	1–58	6
Chronic monocytic leukemia	1	53	19,800	59	None
Reticulum cell sarcoma	2	47, 62	7,500, 8,200	10, 7	2
Lymphosarcoma	7	56–67	3,400–8,800	3–15	5

At least 400 bacteria and 50 cells of a given morphologic type were counted to determine the number of intracellular bacteria that had grains associated. Control cultures containing no leukocytes were processed simultaneously to determine the percentage of extracellular labeled bacteria (usually between 70 and 99%). The results were expressed as the percentage of intracellular bacteria labeled corrected for 100% labeling of extracellular organisms. Background was less than 1 grain per 100 cells.

RESULTS

Mononuclear phagocytes in the circulation of patients with acute myelomonocytic leukemia consisted of monoblasts, promonocytes, and morphologically more differentiated cells resembling normal monocytes. Both promonocytes and monocytes of these patients were glass adherent—a characteristic analogous to that observed in murine myelomonocytic leukemia (2). Immature mononuclear cells had the morphological characteristics described previously (3, 9). For the purpose of this study all mononuclear cells that were glass adherent and phagocytic, regardless of the degree of morphologic differentiation, were grouped together. In general, undifferentiated blast cells were nonadherent.

Phagocytosis. Bacteria were added in great excess to cell monolayers (approximately 200 organisms/cell). After the washing procedure to remove nonphagocytized organisms, only rare extracellular bacteria were observed adherent to the glass slide. By light microscopy the majority of bacteria appeared to be intracellular, with less than 10% of organisms extracellular but adherent to leukocytes.

Because the [^{3}H]thymidine labeling technique is critically dependent upon the ability to distinguish cell-adherent bacteria from bacteria that have been ingested, this point was examined extensively in initial studies previously reported (7, 8). The following approaches were used: (*a*) Intracellular location of phagocytized bacteria was confirmed by electron microscopy, which demonstrated organisms within phagocytic vacuoles. (*b*) Azide and cyanide were used to inhibit intracellular killing without abolishing phagocytosis. With these agents the percentage of intracellular labeled organisms increased. (*c*) Extracellular antibiotics were used to inhibit labeling of extracellular, but not intracellular, bacteria. (*d*) Genetic defects of leukocyte microbicidal mechanisms including myeloperoxidase deficiency and

TABLE II
*Phagocytosis by Mononuclear Phagocytes**

Source of cells	Number of bacteria/cell			
	L. monocytogenes	*Staph. aureus*	*E. coli*	*P. vulgaris*
Normal subjects	1.3±0.7 (11)	4.7±3.7 (15)	1.5±0.4 (4)	2.2±1.3 (11)
Acute myelomonocytic leukemia	4.7±1.2 (5)	5.0±1.6 (6)	4.3±3.2 (4)	3.2±1.9 (7)
Lymphoma	—	3.0 (2)	2.2 (2)	1.2 (2)
Cancer	0.8 (2)	8.5±5.6 (8)	—	1.9±1.0 (4)

* In normal subjects and patients with cancer, the cells enumerated were typical monocytes; in patients with myelocytic leukemia and some patients with lymphoma, the mononuclear phagocytes were of variable morphologic maturity.

chronic granulomatous disease were shown to have abnormally high levels of intracellular labeled bacteria.

The number of bacteria phagocytized by normal monocytes increased linearly with time during the first 60 min of incubation. After 30 min the mean number of organisms/cell varied between one and five (Table II).

The mononuclear phagocytes of normal subjects, patients with acute myelomonocytic leukemia, lymphosarcoma, and one patient with reticulum cell sarcoma appeared to be comparable in their ability to phagocytize the species of bacteria tested (Table II). There was, however, considerable heterogeneity in the malignant leukocyte population, and many leukemic mononuclear cells had bacteria adherent to their surface but were not phagocytic. A similar heterogeneity was not seen among normal monocytes.

Inhibition of bacterial replication; bacterial killing. The principle of the [^{3}H]thymidine technique is that replicating bacteria—whether intracellular or extracellular—incorporate radioactive isotope and can be identified by autoradiography (7, 8). Consequently, the ability of individual types of phagocytic cells to inhibit intracellular bacterial replication can be assessed in a mixed population of leukocytes. As shown in Table III, neutrophils and monocytes from normal subjects inhibited 90% or more of the four test bacteria that had been phagocytized and exposed to tritiated thymidine over a period of 60 min; i.e., 10% or less of intracellular bacteria were labeled. The results of labeling studies of bacteria phagocytized by mononuclear phagocytes from

TABLE III

Labeling by [^{3}H]Thymidine of Organisms Phagocytized by Normal Neutrophils and Monocytes

	% Intracellular bacteria labeled	
	Neutrophils (mean ±2 SD)	Monocytes (mean ±2 SD)
L. monocytogenes	4.8±4.0	7.3±6.6
Staph. aureus 502A	2.0±1.9	5.6±5.4
E. coli	10.4±9.8	9.3±5.0
P. vulgaris	7.1±7.0	8.8±6.6

TABLE IV

[^{3}H]Thymidine Measurement of Intracellular Bacteriostasis by Normal and Malignant Mononuclear Leukocytes

Subjects	Leukocytes/mm^3	MNL*	Chemotherapy‡	Intracellular labeled organisms§			
				L. monocytogenes	*Staph. aureus*	*E. coli*	*P. vulgaris*
Normals (15)			None	7.3±6.6	5.6±5.4	9.3±5.0	8.8±6.6
Acute myelomonocytic leukemia							
1.	4,400	28	AraC, 6TG	14.6	4.3	29.1	18.5
2.	2,200	43	Daun	40	4.1	—	—
3.	5,100	9	Vcr, Mtx	—	2.4	15.8	6.7
4.	18,400	27	None	—	5.3	25.0	—
5.	11,200	17	None	15.8	2.1	60.1	34.7
6.	14,300	12	None	11.5	2.8	—	12.9
7.	2,500	1	AraC, 6TG	16.8	8.4	—	26.0
8.	2,100	18	AraC, 6TG	4.2	1.7	3.6	6.6
9.	33,000	28	None	7.5	—	19.0	17.2
10.	8,900	58	Daun, Vcr	39.0	26.0	—	—
Chronic monocytic leukemia							
11.	19,800	59	None	—	4.9	5.6	3.1
Lymphomas							
12. LSA‖	8,500	2	Vcr, Pred	—	12.0	61.2	29.2
13. RCS¶	7,500	10	Cycloph	8.2	26.0	—	—

* MNL, mononuclear leukocytes, including all identifiable monoblasts, promonocytes, and more differentiated cells.

‡ AraC, cytosine arabinoside; 6TG, 6-thioguanine; Daun, daunorubicin; Vcr, vincristine; Mtx, methotrexate; Cycloph, cyclophoshamide.

§ % of labeled organisms was determined after 30 min for phagocytosis and 30 min exposure to [^{3}H]thymidine, as described in Methods. Values for the normal are given as mean ±2 SD.

‖ LSA, lymphosarcoma.

¶ RCS, reticulum cell sarcoma.

TABLE V

Effect of Antibiotics on Staphylococcus aureus 502A, Extracellular and within Defective Mononuclear Phagocytes

Addition	Extracellular viable bacteria	Intracellular bacteria labeled	Intracellular viable bacteria
	colonies/ml	%	*colonies/ml*
Saline	1.4×10^6	17.5	1.8×10^5
Streptomycin, 50 μg/ml	2.3×10^4	13.0	1.6×10^5
Gentamicin, 2 μg/ml	$<1.0 \times 10^2$	13.3	1.4×10^5

patients with disease are shown in Table IV. The results shown are those for a single time point (60 min). When the time period for phagocytosis and labeling was increased to 90 min, a similar pattern was observed. However, when the period was increased to 120 min, both digestion of some organisms and intracellular replication of others were apparent (8), rendering difficult the interpretation of results.

Additional information necessary for the interpretation of the data in Table IV is the relationship between the number of organisms phagocytized and the number labeled by [^{3}H]thymidine. In initial studies it was observed that the percentage of intracellular organisms labeled was constant up to a total of approximately 30 bacteria/cell. Above 30 organisms the percentage labeled increased, suggesting that the bacteriostatic capacity of the leukocyte was exceeded. In the present study the number of phagocytized organisms rarely exceeded 10/cell (Table II).

Of 10 patients with acute myelomonocytic leukemia, only two (nos. 6 and 8) had leukocytes that inhibited all test organisms normally. The mononuclear cells of the eight remaining patients inhibited the intracellular replication of at least one bacterial species abnormally (i.e., more than two standard deviations beyond the normal mean), and five of the patients had leukocytes that inhibited two or more of the test species abnormally. Three of the patients were studied on three or more occasions over a period of 2 wk. Abnormalities of leukocyte function were consistent over that time period.

Abnormalities of mononuclear leukocyte function were more often observed with *L. monocytogenes* (a facultative intracellular parasite) and the gram-negative organisms than with *Staphylococcus aureus*. At the time of study, six of the patients with acute myelomonocytic leukemia were receiving therapy and four of the patients were receiving no drug treatment. Monocytes from three of the four untreated patients were defective against at least one of the test organisms. Therefore, reduced microbicidal inhibitory activity of mononuclear phagocytes in acute myelomonocytic leukemia could not be ascribed solely to drug treatment.

There was no apparent correlation between the height of the white blood count or the percentage of mononuclear leukocytes and bacteriostatic function. All the patients in this group had serious bacteriologic infection sometime during the course of their illness.

The mononuclear phagocytes of the patient with chronic monocytic leukemia were normal in their ability to inhibit all three of the organisms tested.

Of the nine patients with lymphoma, six with lymphosarcoma and one with reticulum cell sarcoma were entirely normal in the bacterial labeling studies. Monocytes from one patient with reticulum cell sarcoma receiving chemotherapy were abnormal when tested against *Staph. aureus* but normal when tested against *L. monocytogenes* (Table IV). Monocytes from another patient with lymphosarcoma were abnormal when tested against *Staph. aureus, Escherichia coli,* and *Proteus vulgaris* (Table IV). She also had defective neutrophil function. Her abnormality persisted over the course of 1 mo when she was receiving vincristine as her sole therapy. She ultimately died of fulminant gram-negative bacterial sepsis.

Of the 37 microbial inhibitory tests of monocytes from patients with nonhematologic cancer, only one was outside the normal range. Seventeen of these tests were on patients receiving chemotherapy at the time of study. It was concluded that malignancy or cytotoxic drug therapy *per se* was not a cause of an abnormal leukocyte functional test.

In the standard bacterial killing assay based on viable colony counts (6), the neutrophil is the principal phagocyte and often obscures the microbicidal contribution of other phagocytic cells (10). One of our patients (no. 10, Table IV) with myelomonocytic leukemia had no circulating neutrophils and sufficient phagocytic mononuclear cells to perform a standard killing assay with *Staph. aureus* 502A. Her mononuclear cells killed 10 and 60% of phagocytized bacteria at 30 and 60 min, respectively. These values are lower than those reported for normal monocytes (11, 12). She was one of the few patients who demonstrated an abnormally high degree of labeling of intracellular *Staph. aureus* by [^{3}H]thymidine.

Protection from extracellular antibiotics. Antibiotic-sensitive organisms outside the cells are readily killed when exposed to even low concentrations of appropriate antibiotics. Killing may be measured by loss of viable colony counts or by inhibition of [^{3}H]thymidine labeling (Table V). When such organisms are phagocytized by defective mononuclear cells, they appear to be protected

from the bactericidal action of the antibiotics. For example, as shown in Table V (one experiment of three), mononuclear phagocytes from a patient with myelomonocytic leukemia were defective in their ability to kill bacteria. Addition of antibiotics to the culture medium inhibited extracellular bacteria but had no effect on replicating intracellular organisms. The ingested organisms were therefore protected by virtue of their intracellular residence.

DISCUSSION

Analysis of normal and malignant mononuclear cell differentiation in man and the mouse suggests the following maturational sequence: promonocyte-monocyte-tissue macrophage (13, 14). In these species and probably in many mammalian species, receptors on the cell surface for IgG immunoglobulins are detectable in the earliest identifiable cells of the series (15). Therefore, a mechanism exists for attachment and subsequent ingestion of opsonin-coated bacteria. Phagocytic capacity increases with progressive cellular maturation and is best expressed in the mature macrophage (13). The bactericidal mechanisms of the mature mononuclear phagocytes are largely undefined; they appear to be at least in part dependent upon available oxygen (4) and independent of the enzyme, myeloperoxidase, which does not exist in the mature cell. In contrast, monocytes depend upon myeloperoxidase for killing at least some microbial species (16).

From the results reported here, there appears to be a disparity between the development of the phagocytic process and the bacterial killing mechanism of the mononuclear phagocytes of some patients with acute myelomonocytic leukemia and lymphoma. In normal monocytes, phagocytic ability and capacity to inhibit intracellular replications are well balanced, and over 90% of the test organisms are inhibited or killed in a short time. In the defective malignant mononuclear cells, phagocytosis occurs but intracellular bacterial replication continues to a greater degree than that observed in normal mononuclear leukocytes.

The clinical implications of these findings are that pathogenic bacteria and opportunistic pathogens may be sequestered within the malignant mononuclear cell, but not killed. In this cellular sanctuary they may be protected from the effects of antibiotics. This phenomenon, as well as the paucity of normal granulocytes, may contribute to the frequency of microbial infections in acute leukemia of adults and may explain the antibiotic resistance of such infections despite in vitro antibiotic sensitivity of the infecting organism. Other factors contributing to the enhanced susceptibility to infection in acute leukemia include immunologic impairment associated with chemotherapy and decreased numbers of circulating neutrophils. Additionally, the small numbers of mature neutrophils may be defective in microbicidal activity. Several of the patients described in this report were found to have defect in neutrophil (8), as well as mononuclear leukocyte, function. Together these abnormalities constitute significant impairment of host defense functions.

The observation that microorganisms may be protected from the microbicidal systems of serum and from the lethal effects of antibiotics as a consequence of residence in defective leukocytes is not unique to this report. Klebanoff (17) has recently summarized the evidence for the occurrence of such a phenomenon in patients with a variety of hereditary leukocyte defects.

In addition to the abnormalities of phagocyte function in certain hematologic malignancies reported here, defective monocyte microbial activity has also been described in chronic granulomatous disease (11, 12), in children with chronic neutropenia (18), and in myeloperoxidase deficiency (5, 16).

The mechanism of this abnormal function in malignant hematologic disease is entirely obscure. Its elucidation must await better understanding of the microbicidal process in normal mononuclear leukocytes. The bactericidal mechanisms of mononuclear phagocytes may be different from those of neutrophils (19), and discovery of additional syndromes of defective monocyte function may be anticipated.

ACKNOWLEDGMENTS

I wish to acknowledge the excellent technical assistance of Miss Maria Chan.

This work was supported by U. S. Public Health Service Grant CA 12822.

REFERENCES

1. Warner, N. L., M. A. S. Moore, and D. Metcalf. 1969. A transplantable myelomonocytic leukemia in BALB/c mice: cytology, karyotype, and muramidase content. *J. Natl. Cancer Inst.* **43**: 963.
2. Cline, M. J., and D. Metcalf. 1972. Cellular differentiation in a murine myelomonocytic leukemia. *Blood.* **39**: 771.
3. Cline, M. J., and D. W. Golde. 1973. A review and re-evaluation of the histiocytic disorders. *Am. J. Med.* In press.
4. Cline, M. J. 1970. Bactericidal activity of human macrophages: analysis of factors influencing the killing of *Listeria monocytogenes*. *Infect. Immun.* **2**: 156.
5. Lehrer, R. I., and M. J. Cline. 1969. Leukocyte myeloperoxidase deficiency and disseminated candidiasis: the role of myeloperoxidase in resistance to Candida infection. *J. Clin. Invest.* **48**: 1478.
6. Hirsch, J. C., and B. Strauss. 1964. Studies on heat-labile opsonin in rabbit serum. *J. Immunol.* **92**: 145.
7. Cline, M. J. 1972. Microbicidal activity of human eosinophils. *J. Reticuloendothel. Soc.* **12**: 332

8. Cline, M. J. 1972. A new white cell test which measures individual phagocyte function in a mixed leukocyte population. I. A neutrophil defect in acute myelocytic leukemia. *J. Lab. Clin. Med.* **81**: 311.
9. Golde, D. W., and M. J. Cline. 1973. Growth of human bone marrow in liquid culture. *Blood.* **41**: 45.
10. Mickenberg, I. D., R. K. Root, and S. M. Wolff. 1972. Bactericidal and metabolic properties of human eosinophils. *Blood.* **39**: 67.
11. Davis, W. C., H. Huber, S. D. Douglas, and H. H. Fudenberg. 1968. A defect in circulating mononuclear phagocytes in chronic granulomatous disease of childhood. *J. Immunol.* **101**: 1093.
12. Rodey, G. E., B. H. Park, D. B. Windhorst, and R. A. Good. 1969. Defective bactericidal activity of monocytes in fatal granulomatous disease. *Blood.* **33**: 813.
13. Cohn, Z. A. 1968. The structure and function of monocytes and macrophages. *Adv. Immunol.* **9**: 163.
14. van Furth, R., and M. M. C. Diesselhoff-Den Dulk. 1970. The kinetics of promonocytes and monocytes in the bone marrow. *J. Exp. Med.* **132**: 813.
15. Cline, M. J., and M. A. S. Moore. 1972. Embryonic origin of the mouse macrophage. *Blood.* **39**: 842.
16. Lehrer, R. I. 1972. The fungicidal activity of human leukocytes. *In* Phagocytic Mechanisms in Health and Disease. R. C. Williams, Jr., and H. H. Fudenberg, editors. Intercontinental Medical Book Corporation, New York. 151.
17. Klebanoff, S. J. 1971. Intraleukocytic microbicidal defects. *Annu. Rev. Med.* **22**: 39.
18. Baehner, R. L., and R. B. Johnston, Jr. 1972. Monocyte function in children with neutropenia and chronic infection. *Blood.* **40**: 31.
19. Thalinger, K. K., and G. L. Mandel. 1971. Bactericidal activity of macrophages in an anaerobic environment. *J. Reticuloendothel. Soc.* **9**: 393.