The Role of the Phagocyte in Host-Parasite Interactions

I. The Phagocytic Capabilities of Leukocytes from Lymphoproliferative Disorders*

ANTHONY J. Sbarra, WILLIAM SHIRLEY, RATNAM J. SELVARAJ,† EIETSU OUCHI,† AND ERNEST ROSENBAUM‡

(Department of Pathology and Medical Research, St. Margaret's Hospital; and Department of Obstetrics and Gynecology, Tufts University School of Medicine, Boston, Massachusetts)

SUMMARY

The phagocytic and killing capabilities of leukocytes from a number of lymphoproliferative disorders have been studied. For comparative purposes particle uptake rates (phagocytic rates) and subsequent killing rates (bactericidal rates) have been determined on a series of normal (control) leukocyte populations. These data have been found to vary with the bacteria-to-phagocyte ratio. By use of the method of least squares a line of regression was calculated over a selected bacteria-to-phagocyte ratio. The standard error of the estimates was calculated, and arbitrarily two standard errors of the mean were established as a trend boundary. Thus any subsequent result that did not fall within this boundary was considered abnormal, and conversely any result that did fall within the boundary was considered normal.

According to this standard it has been shown that a decreased phagocytic rate and subsequent bactericidal rate are characteristic of some of the lymphoproliferative disorders studied. By substituting normal serum for autologous serum in the phagocytic system many abnormal leukocyte clearance patterns returned to normal. This would suggest the participation of altered serum factors in the decreased phagocytic capability which was observed. However, the presence of a modified leukocytic function is also indicated by some of the results. In most cases patients with abnormal clearance patterns also show some clinical complications. The converse, however, is not true. These complications are discussed in relation to the abnormal clearance patterns observed.

Also, the data suggest that, generally, leukocytes from patients undergoing steroid (prednisone) therapy have a decreased bactericidal activity.

It is known that some patients with lymphoproliferative disorders are more susceptible to repeated infections than would normally be expected (1, 10, 15). The factors responsible for this increased susceptibility have not, however, been completely elucidated. Alterations in both the humoral and cellular defenses of the host have been considered as at least contributing to this increased susceptibility. For example, the low γ-globulin levels found in some leukemias have been implicated by many workers as contributory to infection in the chronic lymphocytic leukemias and lymphosarcomas (5, 7, 11, 18). Studies on the contribution of cellular factors to the observed increased susceptibility of leukemias to infection have been confined mainly to the capacity of the circulating leukocytes to engulf microorganisms. These studies with few exceptions have indicated that the phagocytic activity of leukocytes from the lymphoproliferative disorders is normal, although no such agreement is available for other types of leukemias (2, 6, 16, 17). Phagocytosis has been restricted primarily to observations of particle uptake, and it has been monitored by determining the "phagocytic index" (i.e., percentage of cells phagocytizing and/or number of bacteria engulfed per cell). The additional and equally important functional parameter—the fate of the ingested organism—has inexplicably received far less attention.

The present report is concerned with the phagocytic
capabilities of leukocytes obtained from patients with lymphoproliferative disorders such as lymphosarcoma and chronic lymphocytic leukemia. The nonlymphoproliferative disorders and other diseased states were also studied and will be reported in a subsequent publication. Both uptake and killing capacities of the leukocytes and the effect of serum on these functions were studied, as well as their relation to the clinical picture. For testing the phagocytic and bactericidal rates we have employed a system consisting of buffy coat leukocytes and autologous or normal serum, with *Escherichia coli*, *Pseudomonas aeruginosa*, and/or *Staphylococcus albus* as test bacteria.

**MATERIALS AND METHODS**

Human leukocytes from both normal hospital personnel (20–70 yr. of age) and patients with various diseased states were obtained at St. Margaret's Hospital and through the courtesy of Dr. William Dameshek and Dr. Roger Daniels, Blood Research Laboratory, of the New England Center Hospital. The cells were obtained by filling with blood a 50-ml syringe containing 2.5 ml of 20 per cent dextran and 1.5 ml heparin (1000 units/ml). The syringe was allowed to stand in a vertical position for 45 minutes at 4°C. Theuffy coat was transferred into a centrifuge tube through a bent needle and further purified by quickly passing through it the plunger of a Teflon homogenizer rotating at low speed 2–4 times, by the method of Christlieb, Sbarra, and Bardawil (3). Leukocytes obtained by this method usually contained less than one erythrocyte per leukocyte. However, the homogenization procedure resulted in an isolate that varied somewhat from the peripheral blood differential picture—often containing a higher percentage of polymorphonuclear neutrophils. After isolation a total and differential white cell count was performed by conventional methods. The cells were then brought to the desired concentration in bovine albumin fraction V (HGB) (4, 9). Aseptic conditions were maintained throughout the procedures. The time elapsed from drawing the blood to use was approximately 2 hours.

*S. albus*, *E. coli*, and/or *P. aeruginosa* served as the test bacteria, and they were obtained from the stock culture collection at St. Margaret's Hospital. Overnight cultures of the bacteria, grown in trypticase soy broth, were washed twice in HBG and standardized to a known concentration (Klett units 110 [540 mp] in a Klett-Summerson test tube model colorimeter equaling approximately 4 × 10⁸ organisms/ml). Serum was obtained by allowing an additional 10 ml of blood from the patient to clot at room temperature for 30 minutes. The clot was rimmed and the serum collected after centrifugation. Normal serum was collected in the same manner from a group of hospital personnel on the same day the cellular isolates from the patients were tested. Since the strain of *E. coli* used was readily lysed by fresh human serum, it was necessary to inactivate it at 56°C for 30 minutes prior to use. This treatment was found not to interfere with the opsonizing activity of the serum.

The experimental procedure for studying phagocytic and bactericidal rates was essentially that of Maaløe (8) and Cohn and Morse (4). The reactions were carried out in two series of siliconized test tubes consisting of 0.2 ml of a bacterial suspension of known concentration, 0.2 ml of autologous or normal serum, and 0.6 ml of HBG. After 5 minutes' equilibration in a water bath (37°C) 1 ml of the cellular suspension was added to one series of tubes and 1 ml of HBG to the other series. The latter served as the bacteria-serum control. The tubes were then incubated in a vertical revolving drum at ½ r.p.m. on a metabolic (circular rotation, 100 r.p.m.) shaker in a warm room (37°C). An attempt was made to keep the bacteria-to-phagocyte ratio optimal, approximately 3:8:1 for *S. albus*, 2:5:1 for *E. coli*, and 3:8:1 for *P. aeruginosa*. This was not always feasible, however, since different amounts of blood of varying composition were obtained from the patients. At selected time intervals an experimental and control tube were removed, and a 0.2-ml aliquot was diluted to 2 ml with trypticase soy-broth and homogenized with a Teflon homogenizer. The sample was then diluted in M/15 phosphate buffer and plated with standard techniques used for viable cell counts. This gave the total viable count. Next a 1-ml aliquot from the experimental tube was added to 4 ml HBG, mixed, and centrifuged at 500 r.p.m. for 4 minutes in an International Model UV centrifuge. A 0.2-ml aliquot of the supernatant was homogenized as before, diluted, and plated. This gave the total extracellular bacteria. The entire supernatant was then removed without disturbing the cellular pellet and the pellet resuspended in 1 ml HBG. A 0.2-ml aliquot of the supernatant was again removed and added to 1.8 ml trypticase soy broth, homogenized, and plated, giving the total bacteria associated with the cells.

In some cases the phagocytic system was modified—i.e., to a normal human leukocyte isolate different leukemic serum was added. In these cases the leukocytes were washed once, to reduce autologous serum concentration, before the Teflon homogenization step; this, in effect, resulted in two cellular washes.

The possibility that bactericidal substances (i.e., lysozyme, phagocytin, etc.) would be liberated by the leukocytes during the incubation period and result in extracellular killing was examined. The complete reaction mixture not containing the bacteria was incubated for varying periods of time. The supernatant, after centrifugation was found not to be bactericidal for the test bacteria used.

**RESULTS**

*Bacterial clearance by normal human leukocytes.*—The phagocytic (particle uptake) rates and the bactericidal rates of normal human leukocytes with *S. albus* used as test organism can be seen in Chart 1. Represented are the total viable bacteria in presence of serum only (curve A), the kinetics for total bactericidal activity of the leukocytes and the serum suspension (curve B), supernatant or extracellular bacterial uptake kinetics (phagocytic rate, curve C), and bacteria associated with the

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1 Phagocytes are defined herein as those cells with a marked phagocytic capacity such as polymorphonuclear neutrophils, band nuclear neutrophils, monocytes, and eosinophils; lymphocytes, blasts, and other immature forms are not included.
CHART 1.—Clearance kinetics with normal buffy coat leukocytes and *Staphylococcus albus*.

Curves A and B represent total viable bacteria at given time intervals in presence of autologous serum only. Curve B represents total bacteria present with buffy coat leukocytes and serum at given time intervals. Curve C represents total viable bacteria in 500 r.p.m. supernatant—i.e., phagocytic (particle uptake) rate curve; Curve D, the total viable bacteria in 500 r.p.m. sediment fraction associated with the leukocytes. Experimental points are the mean of two typical experiments. Average W.B.C., 14,000/cu mm; percentage phagocytes (i.e., polymorphonuclear neutrophils, band neutrophils, monocytes, and eosinophils), 90; average bacteria:phagocyte ratio 3:1. Normal $T_{00}$ percentage decrease in supernatant or phagocytic rate, 68. $T_{0}$, bacterial kill rate, 68 per cent; and $T_{50}$, bacterial kill rate, 81 per cent. See text for additional details.

CHART 2.—Clearance kinetics with normal buffy coat leukocytes and *Escherichia coli*.

Curves A, B, C, and D are similar to those in Chart 1. Experimental points are the mean of two typical experiments. Average W.B.C., 10,600/cu mm; percentage phagocytes, 82. Average bacteria:phagocyte ratio, 4:1. Normal $T_{00}$ percentage decrease in supernatant or phagocytic rate, over 99; $T_{0}$, percentage bacterial kill, 99; and $T_{50}$, percentage bacterial kill, 99. See text for additional details.

CHART 3.—Clearance kinetics with normal buffy coat leukocytes and *Pseudomonas aeruginosa*.

Curves A, B, C, and D are similar to those in Chart 1. Experimental points are the mean of two typical experiments. Average W.B.C., 9,300; percentage phagocytes, 91. Average bacteria:phagocyte ratio, 4:1. Normal $T_{00}$ percentage decrease in supernatant or phagocytic rate, 97; $T_{0}$, percentage bacterial kill, 88; $T_{50}$, percentage decrease in supernatant or phagocytic rate, over 99; $T_{50}$, percentage bacterial kill, 96. See text for additional details.

cellular pellet (curve D). From these clearance data (curves A–C) normal phagocytic and bactericidal rates can be calculated. For example, the 60-minute phagocytic rate can be determined by dividing the decrease in number of bacteria in the extracellular supernatant (curve C) during that incubation period by the time zero supernatant count. Also, bactericidal rates can be determined by dividing the decrease in total bacteria (curve B) present after incubation by the bacteria present at the start of the experiment. Chart 2 gives data for two typical experiments with *E. coli* as test organism and Chart 3 for two experiments with *P. aeruginosa* as test organism.

To confirm the extent of bacterial uptake direct microscopic counts were performed at all times tested in a number of different experiments. At $T_{0}$ approximately 2–3 per cent of the initial bacterial population was found to be associated with the cellular pellet (curve D). Light microscopy of this pellet at $T_{0}$ revealed leukocytes essentially free of intracellular bacteria. At subsequent time intervals, direct microscopic observation of the pellet revealed that over 90 per cent of the phagocytes had engulfed bacteria.

To establish clearance kinetics and test assay reproducibility (*S. albus* as the test organism) the phagocytic and bactericidal rates were calculated for twelve different samples of leukocytes obtained from apparently healthy hospital personnel; they were found to be primarily dependent on the bacteria to phagocyte ratio. This series of twelve samples included bacteria:phagocyte ratios with ranges of 3–15:1 and the absolute number of phagocytes ranging from 5,000/cu mm to 16,000/cu mm. From these normal results a line of regression was calculated according to the least squares method for some of the comparative kinetics previously described. Chart 4 shows the regression line for the phagocytic rate at 3 hours, with *S. albus* as test organism and varying bacteria:phagocyte ratio.
CHART 4.—Phagocytic rates at 1 hour with Staphylococcus albus as test organism.
Line X: Mean regression line \( (Y = 89.2 - 0.9X) \) derived from phagocytic rates at different bacteria to phagocyte ratios. Lines Y and Z: Trend boundaries of two standard estimates of the mean regression line \( (\text{Syx} = \pm 9.5) \), line Z is 100 or maximum rate. Circles outside of trend boundaries indicate cases with abnormal activity. Cases with normal activity fall within trend boundaries and are not shown. See text for additional details.

CHART 5.—Phagocytic rates at 30 minutes with Escherichia coli as test organism.
Line X: Mean regression line \( (Y = 95 + 0.8X) \) derived from phagocytic rates at different bacteria to phagocyte ratios. Lines Y and Z: Trend boundaries of two standard estimates of the mean regression line \( (\text{Syx} = \pm 0.5) \); line Z is 100 or maximum rate. Circles outside of trend boundaries indicate cases with abnormal activity. Cases with normal activity fall within trend boundaries and are not shown. See text for additional details.

CHART 6.—Phagocytic rates at 30 minutes with Pseudomonas aeruginosa as test organism.
Line X: Mean regression line \( (Y = 100.1 - 1.26X) \), derived from phagocytic rates at different bacteria to phagocyte ratios. Lines Y and Z: Trend boundaries of two standard estimates of the mean regression line \( (\text{Syx} = \pm 4.7) \). Line Z is 100 or maximum rate. Circles outside of trend boundaries indicate cases with abnormal activity. Cases with normal activity fall within trend boundaries and are not shown. See text for additional details.

should be noted that the upper trend boundary would fall above the 100 per cent level and thus is not shown. However, line Z showing the 100 per cent level is extended from the ordinate for convenience.

Bacterial clearance patterns of leukocytes from lymphoproliferative disorders.—Table 1 presents clinical and experimental data of the 37 cases studied. The data were derived from the buffy coat isolates of 26 patients with lymphosarcoma or chronic lymphocytic leukemia. For quick reference the abnormal cases in this table are marked with an asterisk. Further, the cases with abnormal phagocytic rates are represented as solid circles in Charts 4–6, and it can easily be seen that these statistics fall outside of the trend boundaries established and thus suggest some abnormality.

Of the 37 cases studied (i.e., 26 patients) with lymphoproliferative disorders, eleven cases (cases 1A, 1B, 4, 7A, 8, 9B, 18B, 19A, 19B, 21, and 23) showed no deviation from the normal picture. The eleven cases with normal patterns of phagocytic and bactericidal capability rarely showed any abnormal clinical complications. The normal results with cases 7A, 9B, and 18B were inconsistent with the abnormal patterns obtained on repeat tests of these patients. Case 7A was tested 1 year before the abnormal results were obtained (case 7B). It was not known at the time of first testing whether the patient was hypogammaglobulinemic, and no complications were present at that time. Case 9B, although statistically normal, has shown enhanced uptake of E. coli when normal serum was substituted for autologous serum. The patient was hypogammaglobulinemic but had no history of any of the usual leukemic complications. Case 18B was normal with no complications at time of testing, but case 18A was unusual and will be discussed.

Twenty-six cases, on the other hand, showed an abnormal pattern of reduced phagocytic and bactericidal abilities. In all but three of these cases some clinical variation from normal such as susceptibility to infection, pronounced fatigue, known cryoglobulinemia, or known hypogammaglobulinemia was noted. Both reduced phago-
<table>
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<th>Case no., age, sex</th>
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<th>Treatment</th>
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<th>Remarks</th>
<th>PLC</th>
<th>% Fig. 1</th>
<th>Remarks</th>
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<th>E. coli, normal serum</th>
<th>P. aeruginosa, autologous serum</th>
<th>P. aeruginosa, normal serum††</th>
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</table>
| 1A               | Lymphosarcoma (4 yr.)             | Prednisone, 25 mg/day | 9,700 | Out-patient | 91 | 17,200 | 5:1  
| M 60             |                                   |           |               |         |     |         |         | 94      | 90                       | 90                       |                             |                             |
| 1B               | Lymphosarcoma (4 yr.)             | Prednisone, 15 mg/day; Leukeran, 8 mg/day | 5,200 | Out-patient | 81 | 9,000 | 12:1   
| M 60             |                                   |           |               |         |     |         |         | 83      | 54                       | 67                       |                             |                             |
| 2A††             | Lymphosarcoma (4 yr.)             | Prednisone, 20 mg/day; 4 units blood; 2 units packed red cells previously | 7,720 | Recurrent infections | 20 | 4,300 | 6:1  
| M 61             |                                   |           |               |         |     |         |         | 63      | 0                        | 0                        |                             |                             |
| 2B††             | Lymphosarcoma (4 yr.)             | None      | 5,800 | Recurrent infections; ambulatory | 53 | 5,900 | 9:1  
| M 61             |                                   |           |               |         |     |         |         | 58      | 39                       | 35                       | 56                          |                             |
| 3††              | Giant follicular lymphosarcoma (3 mo.) | Prednisone | 6,950 | Hospitalized | 63 | 5,500 | 13:1  
| F 62             |                                   |           |               |         |     |         |         | 83      | 69                       | 31                       |                             |                             |
| 4                | Lymphosarcoma (1 yr.)             | None      | 7,550 | Out-patient | 78 | 9,300 | 13:1 4:1   
| F 62             |                                   |           |               |         |     |         |         | 82      | —                        | 81                       | 84                          | 99                          |
| 5A††             | Lymphosarcoma (5 mo.)             | None      | 12,000 | Chronically fatigued for 5 yr.; out-patient | 12,100 | 12:1 5:1  
| M 60             |                                   |           |               |         |     |         |         | 37      | 29                       | 66                       |                             |                             |
| 5B††             | Lymphosarcoma (2 yr.)             | None      | 6,050 | A slow healer; hypogammaglob.; out-patient | 35 | 12,200 | 3:1 3:1 8:1   
| M 61             |                                   |           |               |         |     |         |         | 47      | 31                       | 99                       |                             |                             |
| 6A††             | Lymphosarcoma (5 yr.)             | Prednisone, 2.5 mg/day | 6,400 | Autoimmune hemolytic anemia, hospitalized | 88 | 14,000 | 3:1  
| F 66             |                                   |           |               |         |     |         |         | 94      | 65                       | 65                       |                             |                             |
| 6B††             | Lymphosarcoma (5 yr.)             | Prednisone, 2.5 mg/day | 8,200 | Out-patient | 79 | 5,500 | 6:1 6:1 5:1   
| F 66             |                                   |           |               |         |     |         |         | 95      | 0                        | 0                        | 86                          |                             |
| 7A               | Lymphosarcoma (8 yr.)             | None      | 3,200 | Out-patient | 78 | 7,100 | 3:1  
<p>| F 35             |                                   |           |               |         |     |         |         | 98      | 99                       | 99                       |                             |                             |</p>
<table>
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<th>Case no., age, sex</th>
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<th>Isolate WBC% Phg.*</th>
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<td>7B†† 35 F</td>
<td>Lymphosarcoma (9 yr.)</td>
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<td>4,100</td>
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<td>7:1</td>
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<td>1:1</td>
<td>1:1</td>
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<td>9A†† 45 F</td>
<td>Lymphosarcoma (15 mo.)</td>
<td>None</td>
<td>7,500</td>
<td>Abdominal mass; hospitalized; hypogammaglobulinemia</td>
<td>6,300</td>
<td>5:1</td>
<td>8:1</td>
<td>9:1</td>
<td>11:1</td>
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<td>9B 45 F</td>
<td>Lymphosarcoma</td>
<td>8 mg. leukenan</td>
<td>2,800</td>
<td>Out-patient</td>
<td>4,350</td>
<td>7:1</td>
<td>7:1</td>
<td>9:1</td>
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<td>7,600</td>
<td>Abdominal mass; hypogammaglobulinemia; out-patient</td>
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<td>3,850</td>
<td>Hospitalized</td>
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<td>5:1</td>
<td>5:1</td>
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<td>12A†† 64 M</td>
<td>Lymphosarcoma (2 yr.)</td>
<td>15 mg. prednisone</td>
<td>12,600</td>
<td>Slight hypogammaglobulinemia; out-patient</td>
<td>5,600</td>
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<td>5 mg. prednisone</td>
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<td>Slight hypogammaglobulinemia; hospitalized</td>
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<td>6:1</td>
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<td>13†† 62 F</td>
<td>Lymphosarcoma</td>
<td>30 mg. prednisone</td>
<td>11,400</td>
<td>Hospitalized</td>
<td>7:1</td>
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<td>Remarks</td>
<td>Isolate WBCI % Fig.†</td>
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<td>14†† 54 F</td>
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<td>8 mg. Leukeran, 15 mg. prednisone</td>
<td>5,150 68</td>
<td>Cellulitis, cryoglobulinemia with hypergammaglobulinemia; hospitalized</td>
<td>5,550 94</td>
<td>6:1 99</td>
<td>6:1 99</td>
<td>8:1 33</td>
<td>99 99</td>
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<td>Massive pleural effusion of rt. side</td>
<td>6,650 92</td>
<td>4:1 99</td>
<td>4:1 99</td>
<td>10:1 71</td>
<td>99 99</td>
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<td>17†† 60 F</td>
<td>Chronic lymphocytic leukemia</td>
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<td>—</td>
<td>Hospitalized</td>
<td>21,000 7:1</td>
<td>50 99</td>
<td>38 99</td>
<td>43 99</td>
<td>99 99</td>
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<td>Prednisone, 25 mg/day; Leukeran 8 mg/day</td>
<td>600,000 1</td>
<td>Recurrent hospitalization for infection; out-patient</td>
<td>902,000 12:1</td>
<td>90 99</td>
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<td>34,000 32</td>
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<td>94 99</td>
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<td>Prednisone, 75 mg/day</td>
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<td>Out-patient</td>
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<td>99 99</td>
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<td>8,000 5:1</td>
<td>59 99</td>
<td>76 99</td>
<td>40 99</td>
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</tr>
</tbody>
</table>
SBARRA et al.—Phagocytic Capabilities of Leukocytes

1965

TABLE 1—Continued

<table>
<thead>
<tr>
<th>Case no., age, sex</th>
<th>Diagnosis, duration from diagnosis</th>
<th>Treatment</th>
<th>PLC* % Phg.†</th>
<th>Remarks</th>
<th>Isolate WBC % Phg.†</th>
<th>S. albus, autologous serum</th>
<th>E. coli, autologous serum</th>
<th>E. coli, normal serum</th>
<th>P. aeruginosa, autologous serum</th>
<th>P. aeruginosa, normal serum ↑</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>Chronic lymphocytic leukemia (6 mo.)</td>
<td>None</td>
<td>138,000</td>
<td>Out-patient</td>
<td>122,800</td>
<td>11</td>
<td>2:1</td>
<td>99</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>58 F</td>
<td>Chronic lymphocytic leukemia (1.5 yr.)</td>
<td>Prednisone, 15 mg/day</td>
<td>63,400</td>
<td>Out-patient</td>
<td>27,950</td>
<td>33</td>
<td>4:1</td>
<td>92</td>
<td>89</td>
<td>98</td>
</tr>
<tr>
<td>25A††</td>
<td>Chronic lymphocytic leukemia (2 yr.)</td>
<td>None</td>
<td>218,000</td>
<td>Hospitalized</td>
<td>185,500</td>
<td>9</td>
<td>3:1</td>
<td>45</td>
<td>48</td>
<td>45</td>
</tr>
<tr>
<td>46 M</td>
<td>Chronic lymphocytic leukemia (2 yr.)</td>
<td>100 mg. prednisone</td>
<td>242,000</td>
<td>Hospitalized</td>
<td>169,000</td>
<td>20</td>
<td>2:1</td>
<td>82</td>
<td>74</td>
<td>72</td>
</tr>
<tr>
<td>26B††</td>
<td>Chronic lymphocytic leukemia (5 yr.)</td>
<td>100 mg. prednisone</td>
<td>67,000</td>
<td>Severe hypogammaglobulinemia; recurrent infections previous 2 months; hospitalized</td>
<td>94,300</td>
<td>6:1</td>
<td>6:1</td>
<td>94</td>
<td>91</td>
<td>94</td>
</tr>
</tbody>
</table>

* Peripheral leukocyte count.
† Percentage of total peripheral leukocytes that are considered as phagocytes (polymorphonuclear leukocytes, band neutrophils, monocytes, and eosinophils).
‡ Buffy coat leukocyte count of experimental system.
§ Each block of figures reading from top to bottom represents: (1) ratio of S. albus to phagocyte in phagocytic system (2) 60-minute percentage phagocytic rate (Curve C, Chart 1), (3) 60-minute percentage bactericidal rate (Curve B, Chart 1), (4) 3-hour percentage bactericidal rate (Curve B, Chart 1). See text for additional details.
§§ Each block of figures reading from top to bottom represents: (1) ratio of E. coli to phagocyte in phagocytic system, (2) 30-minute percentage phagocytic rate (Curve C, Chart 2), (3) 30-minute percentage bactericidal rate (Curve B, Chart 2), (4) 60-minute percentage bactericidal rate (Curve B, Chart 2). See text for additional details.
†† Same as § except that fresh serum from apparently healthy laboratory personnel was substituted for autologous serum.
** Each block of figures reading from top to bottom represents: (1) ratio of P. aeruginosa to phagocyte in phagocytic system, (2) 30-minute percentage phagocytic rate (Curve C, Chart 3), (3) 30-minute percentage bactericidal rate (Curve B, Chart 3), (4) 60-minute percentage phagocytic rate (Curve C, Chart 3), (5) 60-minute percentage bactericidal rate (Curve B, Chart 3). See text for additional details.
††† Same as ** except that fresh serum from apparently healthy laboratory personnel was substituted for autologous serum.

Denotes abnormal cases for quick reference.

cytic and bactericidal activity was found with every organism tested in fifteen of the 26 abnormal patterns (cases 2A, 2B, 5A, 7B, 9A, 9C, 12B, 16, 17, 20, 22, 24, 25A, 26B, and 26). Some variation in the phagocytic and bactericidal ability was noted in the other nine abnormal patterns (cases 5B, 6A, 6B, 10, 11, 12A, 13, 14, 15). In this later group, with the exception of cases 6A and 6B, the abnormal pattern was noted with P. aeruginosa as test organism and not with the other bacteria. Four cases of considerable interest are 6A, 6B, 25A, and 25B. Both of these patients on the two times tested showed decreased phagocytic and bactericidal rates with a specific bacteria; with cases 6A and 6B it was E. coli, and with cases 25A and 25B it was P. aeruginosa. Further, by substituting normal serum for autologous serum no increase in uptake was noted (cases 6B, 25A, and 25B).

Some cases with known hypogammaglobulinemia indicated normal capability to phagocytize and kill E. coli when compared with the statistical control. However, by substituting normal serum for autologous serum a considerably enhanced uptake rate was noted. This is shown in Chart 7 for case 5B. It appears that normal
P. aeruginosa can easily be seen when leukemic serum is in the phagocytic system.

In the final two cases, case 3 and case 18A, the abnormality indicated reduced bactericidal rates, though phagocytic rates were normal. Both cases were taking Prednisone at time of testing, and case 18A was also on Leukeran. On the second test 6 months later (case 18B) the patient showed a normal clearance pattern and was not under treatment.

DISCUSSION

The repeated bacterial infections often observed in patients with lymphoproliferative disorders have prompted many investigators to compare the phagocytic capacity of normal human leukocytes with that obtained from hematological disorders (2, 6, 16, 17). Previous studies have been concerned primarily with the capacity of the cell to engulf bacteria by employing phagocytic indexes to determine percentage of leukocytes phagocytizing and/or number of bacteria per leukocyte. Although particle uptake is important, the subsequent fate of the ingested bacteria is perhaps an equally important phagocytic parameter that has previously received little attention. In the present study a method has been employed that measures phagocytic capacity and bactericidal activity simultaneously. Since both phagocytic and bactericidal rates are known to vary with the bacteria-to-phagocyte ratio, a number of experiments were performed at different ratios, and the corresponding normal phagocytic and bactericidal kinetics were obtained. From these experiments the normal linear regressions and standard estimates for different experimental points were determined. With these statistics used as the variation obtained from a normal cell population, differences in phagocytic and bactericidal kinetics have been studied with the phagocyte-serum-bacteria system of some leukemic patients.

The tendency for the lymphoproliferative disorders to show reduced phagocytic and bactericidal rates on different occasions would indicate that this type of disorder does involve the phagocytic system—e.g., polymorphonuclear and band neutrophils, monocytes, eosinophils (not lymphocytes), and serum factors. Of the 37 lymphoproliferative cases studied, 26 showed results that fell outside the normal trend boundaries obtained from normal cell isolates; 24 of these 26 cases showed reduced phagocytosis. These results are not in general agreement with those of previous workers, who studied the phagocytic capabilities of leukocytes from leukemic patients by use of conventional phagocytic indexes (2, 6, 16, 17). These workers (2, 16, 17) showed that the phagocytic function of the mature neutrophil (expressed only as capacity to engulf particles) was normal; however, uniformly "opsonized" bacteria (e.g., preincubation with hyperimmune serum or pooled sera) and whole blood were used in these cases.

Since our system indicated frequent abnormality in particle uptake, and inasmuch as it is known that serum usually promotes particle entry, the contribution of possible abnormal serum factors responsible for the decreased phagocytosis was also tested. By substituting normal serum is more efficient in promoting particle entry and bacterial kill than autologous serum in these patients. To further develop this point serum from two cases with hypogammaglobulinemia were tested with normal cells and P. aeruginosa. The results from one case, 9B, are illustrated in Chart 8. The marked decrease in uptake of

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**Chart 7:** The effect of different sera on the clearance kinetics of leukemic cells with Escherichia coli as test organism. Leukocytic cells and autologous serum were obtained from case 5B. Curves A, B, C and D are similar to those in Chart 1. See text for additional details.

**Chart 8:** The effect of different sera on the clearance kinetics of normal leukocytes and Pseudomonas aeruginosa as test organism. Leukemic serum was obtained from case 9B. Curves A, B, C and D are similar to those in Chart 1. See text for additional details.
serum for autologous serum the phagocytic capability of the leukemic leukocytes was greatly enhanced in all instances tested, with the exceptions of cases 6B, 25A, and 25B. Since many of these cases were known hypogammaglobulinemias, the possibility that low γ-globulin levels and possibly cryoglobulinemia could reduce the activity of the host defenses and lead to increased occurrence of infections must be considered. The decreased phagocytic rates noted when normal leukocytes are exposed to serum obtained from patients with hypogammaglobulinemia lends further support to this line of reasoning.

In the light of these findings it would be interesting to examine the effect of serum or blood transfusion on patients with abnormal clearance kinetics and/or infections. For example, Miller et al. (10) have shown that by injecting γ-globulin to some of their chronic lymphatic leukemia patients with infections an improvement in the infectious picture often resulted. From the data here presented it would not be unreasonable to postulate that the phagocytic defense mechanism in their patients could have returned to normal.

It appears that a patient with hypogammaglobulinemia and decreased phagocytic ability may eventually show an increased susceptibility to infections. This is seen in case 26. This patient was without complications for several years; 2 months previous to testing he had several episodes of infection. When hospitalized for an infection he was found to be severely hypogammaglobulinemic with greatly reduced phagocytic capability; the patient died of bacterial infection shortly thereafter.

However, the possibility that the leukocytes themselves may be altered must also be considered (i.e., cases 6B, 25A, and 25B). Since it has recently been suggested that lysosomes may be involved in killing of ingested microorganisms it is possible that a qualitative and/or quantitative change in the lysosomal structure of the phagocyte has occurred. Experiments designed to explore this and other hypotheses are being conducted. Further, cases 3 and 18A showed unaltered phagocytic rates but clearly indicated reduced bactericidal abilities. This would again suggest the possibilities that the phagocytes themselves might have modified bactericidal capacity, or that serum factors contributing to bactericidal kill are deficient or altered or that the autologous serum in some way is injurious to the cell.

Shaw et al. (14) have shown that the administration of prednisone to patients with chronic lymphocytic leukemia increases the severity of bacterial infections. It has been shown in our laboratory (13) that the serum of patients with chronic lymphocytic leukemia inhibits the respiratory activity of Bacillus subtilis to a much greater extent than do any other sera tested. Further, it has been shown that the sera of these patients is no longer inhibitory if the patients are on prednisone therapy (12). Our present findings with patients 2, 9, and 18 appear to be consistent with the above. These cases were tested at least twice. On the first occasions the patients were on 20-, 100-, or 25-mg, daily doses of prednisone. The resulting clearance statistics in each case were abnormal—a decreased kill was noted. On the second occasions the prednisone was withdrawn, and the resulting clearance patterns either were normal or approached normal. Only patient 25 did not follow the general pattern, but as previously mentioned this case was unusual in several respects. Further, only one of nine cases taking 25 mg. or more of prednisone per day showed a normal clearance pattern, and no case taking 100 mg. or more was normal. It appears that prednisone, at least in these cases, may be interfering with the phagocytic system. Perhaps the steroid is "stabilizing" the lysosomes (19) and thus interferes with availability of lysosomal enzymes for degradation of ingested bacteria. However, the effect of treatment must be studied as an experimental condition and not as a variable in the experiment as in this study.

It is noteworthy that most of the cases indicating an abnormal clearance picture have clinical complications of some degree (Table 1). The converse, however, is not true—i.e., cases with complications do not necessarily show an abnormal clearance picture. At least two explanations may be offered to elucidate this finding. First, the phagocytic defense mechanism per se may not have an important role in these diseased states; second, the phagocytic system of the host will eventually become involved and result in decreased activity and consequently in an increased susceptibility to repeated infections. Obviously, more experiments are needed, as well as repeated studies on individual cases.

It is felt that the approach used in this study has not unequivocally established that the phagocytic system is impaired in patients with lymphoproliferative disease. However, it does appear to support such a thesis. Further, preliminary work with the nonlymphoproliferative disorders indicates that the phagocytic system is not disturbed. These results will be presented in a subsequent publication.

ACKNOWLEDGMENTS

The excellent technical assistance of Miss Lolita Dy Serrano is greatly appreciated. Photography and line drawings by George Daynes and the typing of this manuscript by Mrs. Doris Crocker are gratefully acknowledged.

REFERENCES


The Role of the Phagocyte in Host-Parasite Interactions: I. The Phagocytic Capabilities of Leukocytes from Lymphoproliferative Disorders


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