Bactericidal and Bacteriolytic Activity of Leukemic Sera

Waldemar Pruzanski, Wolf-Dietrich Leers, and Alastair C. Wardlaw

SUMMARY

Bacteriolytic and bactericidal activities against Escherichia coli Lilly were tested in 64 and 56 leukemic sera, respectively. Bacteriolytic activity was lower than normal in acute lymphoblastic and hairy cell leukemia and higher than normal in chronic myelogenous leukemia. No statistically significant abnormality in the bacteriolytic activity was found in acute myeloblastic and in chronic lymphocytic leukemia. In all groups bacteriolytic activity was significantly correlated with the level of serum lysozyme. No correlation with the complement or the immunoglobulin levels was noted.

Out of nine other microorganisms only three gram-negative strains were lysed by leukemic sera, but to a much lesser extent than E. coli Lilly.

In 10 leukemic patients with acute bacterial infection sera were tested for bacteriolytic activity against the causative agents. Only a few sera possessed weak bacteriolytic activity against these microorganisms.

Bactericidal activity was generally higher in leukemic sera than in healthy individuals, especially at a low dilution of serum and a short exposure time.

It is evident that, although bacteriolytic activity of leukemic sera varies greatly, their bactericidal activity is usually normal or enhanced. Therefore, the high incidence of infection in leukemia and the differences in the rate of infections in the high-lysozyme versus low-lysozyme groups cannot be entirely explained on the basis of differences in humoral antibacterial activity.

INTRODUCTION

Acute bacterial infection is a frequent and often fatal complication in leukemia. In spite of this, little is known regarding the causes of susceptibility to infectious agents in this disease. Defense against acute infection in general relies on 2 mechanisms, phagocytosis and serum antibacterial activity. The former mechanism operates through cells capable of engulfing and intracellular killing of microorganisms. The latter is based on humoral bactericidal and bacteriolytic activities against susceptible bacteria. These humoral activities arise from an interaction of several components, of which complement, immunoglobulins, and lysozyme are especially important (24). These 3 factors also serve as specific and nonspecific opsonins (22, 23), thus establishing a link between the cellular and humoral mechanisms.

Normal (12, 19, 20) or low (3, 9, 16, 19, 21) phagocytic activity has been reported in leukemia. Although it was apparent that the high rate of infection in leukemia could not be explained on the basis of depressed phagocytic activity alone (20), no comprehensive investigation of the 2nd defense mechanism, namely humoral antibacterial activity, was undertaken until recently. In 3 early studies of bactericidal activity in leukemia, controversial results have been reported, and no correlation with various serum factors was undertaken (10, 13, 25).

It has recently been noted that the rate of infection was lower (2, 17) and the median survival time longer (2) in leukemic patients with high serum lysozyme. Subsequently, we reported increased bacteriolytic and bactericidal activity in mono- and myelomonocytic leukemia with hyperlysozymemia (18).

In this paper results of the study of bactericidal and bacteriolytic activity of the serum in acute and chronic leukemias, with the exception of previously reported mononuclear types, are reported. These activities were correlated with several serum factors known to participate in antibacterial reactions.

MATERIALS AND METHODS

Sixty-four sera from patients with various forms of active leukemia and 30 sera from healthy individuals, matched by age, were tested for bacteriolytic activity against Escherichia coli Lilly. The breakdown of the leukemic patients was as follows: acute lymphoblastic leukemia, 14; acute myeloblastic leukemia, 19; chronic lymphocytic leukemia, 13; chronic myelogenous leukemia, 16; and hairy cell leukemia, 2. Patients with hematological side-effects of chemotherapy and those with a recent acute infection or any unrelated condition to leukemia, that could change the defence mechanisms of the host, were excluded from the study. None of the patients received antibiotics in the immediate period preceding the assays.

Seventeen leukemic sera and 4 sera of healthy individuals were tested for bacteriolytic activity against 9 other microorganisms. Finally, as a separate group, 10 sera from leukemic patients having an acute bacterial infection were tested for bacteriolytic activity against "autologous" microorganisms isolated from their own body fluids. In each case 2 other sera from patients with a similar type of leukemia...
and 2 sera of healthy individuals were tested for bacteriolytic activity against the isolated microorganism.

Fifty-six sera of leukemic patients and 26 sera of healthy individuals were tested for bactericidal activity against *E. coli* Lilly.

Venous blood was taken under sterile conditions at least 12 hr after the last dose of chemotherapeutic agent was administered to the treated patients. The serum was separated and either used immediately or frozen at −70°C. The majority of the frozen sera were assayed within the 1st month after freezing, being thawed only once, just prior to the experiment. All sera tested for bactericidal activity and many of those used for lytic experiments were checked for bacteriological sterility; all were found to be sterile.

The total hemolytic complement was tested according to the method described by Kabat and Mayer (11). Normal values were estimated in 40 healthy adults. Lysozyme activity was estimated by the lysoplate method of Osserman and Lawlor (15). Pure human lysozyme isolated from the urine of a patient with monocytic leukemia by the method of Alderton et al. (1) was used as a standard. Its purity and the methodology are described in detail elsewhere (18). The concentration of immunoglobulins G, A, and M in the serum was tested by radial immunodiffusion using immunoplates of Hyland Laboratories, Los Angeles, Calif. Normal values were estimated in 100 healthy individuals.

The microorganism used routinely in all bacteriolytic and bactericidal assays was a rough strain of *E. coli* Lilly, particularly sensitive to the lysis by fresh human serum (24). This microorganism was found to be especially suitable for distinguishing between normal and abnormal bacteriolytic and bactericidal activity. Other microorganisms used in a limited number of experiments were *E. coli*, rough strains 011-B4, Hock, and 14325, *E. coli*, smooth strains O55-B5 and 11181, *Proteus vulgaris; Proteus mirabilis; Streptococcus; and Salmonella typhimurium. "Autologous" microorganisms will be described in "Results." The methods of growing the bacteria and preparation for the assays are described elsewhere (18).

Bacteriolytic and bactericidal assays were described in detail previously (18, 24). Briefly, bacteriolytic activity was tested as follows. Tris buffer, pH 8.0, ionic strength 0.06, was used. The test mixture was made up to a total volume of 3.0 ml and the pH of the complete mixture was adjusted to 8.0 and the ionic strength to 0.06. For example, for serum diluted 1/20, the leukemic sera were more active bactericidally than sera of healthy individuals (Chart 1). In 6 instances the activity exceeded that of the controls in more than 2 S.D. of the normal mean. There was a highly significant relationship of the bacteriolytic activity to the serum lysozyme (p < 0.01) (Chart 2) but no such relationship to the level of complement or immunoglobulins.

Bactericidal Activity against *E. coli* Lilly. In dilution 1/10, the leukemic sera were more active bactericidally than sera of healthy individuals (Chart 3). In 6 instances the activity exceeded that of the controls in more than 2 S.D. of the normal mean. In none was it weaker than normal. In dilutions 1/30 and 1/100, however, these differences were not detected, as the leukemic sera had bactericidal activity identical to that of the controls.

**RESULTS**

**Acute Lymphoblastic Leukemia**

Fourteen patients, 7 men and 7 women, were investigated. The age varied from 10 to 79 years with a mean of 34.9. The total hemolytic complement in the serum varied from 158 to 242 HU₉₀/ml² (Table I). The lysozyme ranged from 1.5 to 15.4µg/ml. In 7 sera lysozyme was lower than 5 µg/ml and in only 1 patient did it exceed 15 µg/ml. IgG was low in 3 patients (500 to 580 µg/100 ml), IgA was decreased in 3 cases (48 to 60 µg/100 ml), and IgM was low in 4 sera (22 to 30 µg/100 ml).

**Bacteriolytic Activity against *E. coli* Lilly.** Bacteriolytic activity was in general much below that of the healthy individuals (Chart 1). In 8 of 14 samples at 10-min readings and in 10 of 14 sera at 60-min readings, bacteriolytic activity was below 2 S.D. of the normal mean. There was a highly significant relationship of the bacteriolytic activity to the serum lysozyme (p < 0.01) (Chart 2) but no such relationship to the level of complement or immunoglobulins.

**Bactericidal Activity against *E. coli* Lilly.** In dilution 1/10, the leukemic sera were more active bactericidally than sera of healthy individuals (Chart 1). In 6 instances the activity exceeded that of the controls in more than 2 S.D. of the normal mean. In none was it weaker than normal. In dilutions 1/30 and 1/100, however, these differences were not detected, as the leukemic sera had bactericidal activity identical to that of the controls.

**Acute Myeloblastic Leukemia**

Nineteen patients, 9 women and 10 men, were investigated. The age varied from 19 to 75 years with a mean of 46.2. The total hemolytic complement in the serum varied from 162 to 288 HU₉₀/ml (Table I). The lysozyme ranged from 2.1 to 27.0µg/ml. In 4 sera the lysozyme was below 5.0µg/ml and in 2 it was above 15µg/ml. In only 1 patient was the IgG low, (419 µg/100 ml). IgA was decreased in 2 patients (72 and 82 µg/100 ml) and IgM was low in only 1 serum (29 µg/100 ml).

**Bacteriolytic Activity against *E. coli* Lilly.** The bacteriolytic activity varied from case to case, especially at the early readings up to 30 min. For example, at 10-min reading, 6

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1 The abbreviation used is: HU₉₀, hemolytic units.₉₀
Humoral factors of immunity in leukemic sera

**Table I**

<table>
<thead>
<tr>
<th>Type of leukemia</th>
<th>No. of patients</th>
<th>Lysozyme (µg/ml)</th>
<th>Complement (HU₅₀/ml)</th>
<th>IgG (mg/ml)</th>
<th>IgA (mg/ml)</th>
<th>IgM (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute lymphoblastic</td>
<td>14</td>
<td>5.7 ± 3.8*</td>
<td>201 ± 27</td>
<td>10.22 ± 3.75</td>
<td>2.09 ± 2.33</td>
<td>0.91 ± 0.74</td>
</tr>
<tr>
<td>Acute myeloblastic</td>
<td>19</td>
<td>8.7 ± 5.5</td>
<td>228 ± 40</td>
<td>12.66 ± 4.5</td>
<td>2.50 ± 1.74</td>
<td>1.16 ± 0.89</td>
</tr>
<tr>
<td>Chronic lymphocytic</td>
<td>13</td>
<td>7.8 ± 3.6</td>
<td>208 ± 42</td>
<td>8.30 ± 3.42</td>
<td>1.21 ± 1.06</td>
<td>0.87 ± 0.16</td>
</tr>
<tr>
<td>Chronic myelogenous</td>
<td>16</td>
<td>15.4 ± 7.4</td>
<td>209 ± 38</td>
<td>11.18 ± 4.63</td>
<td>1.32 ± 0.73</td>
<td>0.73 ± 0.43</td>
</tr>
<tr>
<td>Healthy individuals</td>
<td>100</td>
<td>9.7 ± 1.8</td>
<td>167 ± 29*</td>
<td>11.54 ± 2.22</td>
<td>2.33 ± 0.79</td>
<td>1.19 ± 0.42</td>
</tr>
</tbody>
</table>

*Mean ± S.D.

leukemic sera had lytic activity below 2 S.D. of the normal mean of the controls and 7 others had activity above 2 S.D. of the normal mean (Chart 1). At the 30- to 60-min readings the lytic activity was either equal or lower than in the controls. There was a highly significant correlation of the bacteriolytic activity to the level of lysozyme ($p < 0.01$) (Chart 2). There was no correlation with either the complement or the immunoglobulin level.

**Bactericidal Activity against E. coli Lilly.** Fifteen sera were tested for bactericidal activity (Chart 3). In dilution 1/10 at 2-min exposure 7 leukemic sera had bactericidal activity higher than 2 S.D. of the normal mean. In dilution 1/30 and 2-min exposure, only 2 leukemic sera were more active. In dilution 1/100, a longer exposure time was necessary to detect differences between leukemic and normal sera, but the former were still more active on the average.

**Chronic Lymphocytic Leukemia**

Thirteen patients, 10 men and 3 women, belonged to this group. The age varied from 34 to 83 years with a mean of 68.3. The total hemolytic complement ranged from 160 to 268 HU₅₀/ml in 12 patients and was low (116 HU₅₀/ml) in 1 patient (Table 1). The serum lysozyme ranged from 2.8 to 16.9 µg/ml, being lower than 5 µg/ml in 2 sera and higher than 15 µg/ml in 1 serum. Serum IgG was low in 5 sera (420 to 600 mg/100 ml), IgA was low in 5 sera (30 to 62 mg/100 ml), and IgM was low in 6 sera (13 to 30 mg/100 ml).

**Bacteriolytic Activity against E. coli Lilly.** Bacteriolytic activity was on the average slightly higher than that of the healthy individuals; however, the differences were statistically insignificant (Chart 1). The bacteriolytic activity was related to lysozyme level ($p < 0.01$) (Chart 2). The lowest bacteriolytic activity was noted in the serum with low complement (116 HU₅₀/ml) and low lysozyme (5.1 µg/ml). The highest bacteriolytic activity was observed in a sample with the highest lysozyme (16.9 µg/ml) and normal complement.

**Bactericidal Activity against E. coli Lilly.** Eleven sera were tested. In dilutions 1/10 (Chart 3) and 1/30, bactericidal activity was not significantly different from that of healthy individuals. In dilution 1/100 the leukemic sera on the average exhibited weaker activity than those of healthy individuals, but no statistically significant differences were apparent.

**Chronic Myelogenous Leukemia**

Sixteen patients, 10 men and 6 women, belonged to this group. The age varied from 21 to 75 years, with a mean of 44.9. The total hemolytic complement varied from 160 to 320 HU₅₀/ml (Table 1). The serum lysozyme ranged from 5.1 to 32.7 µg/ml, being higher than 15 µg/ml in 7 cases. Low serum IgG was found in 2 sera (420 to 570 mg/100 ml), low IgA in 6 sera (32 to 84 mg/100 ml) and low IgM in 2 sera (16 to 19 mg/100 ml).

**Bacteriolytic Activity against E. coli Lilly.** Bacteriolytic activity of the leukemic sera was higher than of the healthy individuals especially in the 1st 30 min of the reaction (Chart 1). Eleven of 16 sera diluted 1/20 had at 10-min readings bacteriolytic activity higher than 2 S.D. of the normal mean. No such difference was noted at 60-min readings. Bacteriolytic activity was related to the serum lysozyme ($p < 0.01$). (Chart 3).

**Bactericidal Activity against E. coli Lilly.** In dilution 1/10 at 2-min exposure bactericidal activity of the leukemic sera was higher than of the healthy individuals (Chart 3). No differences, however, were noted in the sera diluted 1/30 or 1/100.

**Hairy Cell Leukemia**

There were 2 men aged 50 and 57 years with this type of leukemia. The total hemolytic complement was 208
bacteriolytic activity whatsoever (readings at 60 min less than 10%) was detected against 2 smooth strains of E. coli, rough strains of E. coli, Streptococcus, and S. typhimurium.

Bacteriolytic Activity against “Autologous” Microorganisms. In 10 leukemic patients microorganisms were isolated from various body fluids during acute infections. Then the serum of the patient from which the microorganism was obtained (hence “autologous”), 2 sera from other patients with the same type of leukemia and 2 sera of healthy individuals were tested for bacteriolytic activity against these bacteria.

The results show (Table 3) that in only 4 instances did the sera possess some bacteriolytic activity, i.e., the lysis exceeded 10% at 60-min readings. In the rest, virtually no lysis of the bacteria occurred. It seems, however, that almost all leukemic sera were slightly more active than the controls.

DISCUSSION

Almost every patient with leukemia suffers from at least 1 infectious episode during the course of the disease (18). Furthermore, infection is found at death in more than three-fourths of autopsies of leukemic patients (18). Susceptibility to infection may be caused by a variety of factors, impairing either phagocytosis and/or serum antibacterial activity. It seems that the leukemic process by itself may alter these functions; however, several iatrogenic factors may also contribute to the high rate of infections. Corticosteroids and various chemotherapeutic agents may suppress production of antibodies and reduce the number of immunoglobulin-producing cells and phagocytes.

Of the 2 mechanisms of the resistance to infection, only phagocytosis has been studied in leukemia (3, 9, 12, 16, 19–30).
Table 2

Bacteriolytic activity against gram-negative microorganisms

<table>
<thead>
<tr>
<th>Type of leukemia</th>
<th>No. of patients</th>
<th>Total hemolytic complement (HU/ml)</th>
<th>Lysozyme (µg/ml)</th>
<th>E. coli, rough O11:B4</th>
<th>P. vulgaris</th>
<th>P. mirabilis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute blast cell</td>
<td>5</td>
<td>158-206</td>
<td>5.4-8.2</td>
<td>40.9</td>
<td>56.0</td>
<td>33.3</td>
</tr>
<tr>
<td>Chronic lymphocytic</td>
<td>5</td>
<td>160-224</td>
<td>4.2-10.5</td>
<td>53.3</td>
<td>44.9</td>
<td>20.1</td>
</tr>
<tr>
<td>Chronic myelogenous</td>
<td>5</td>
<td>168-230</td>
<td>5.1-21.6</td>
<td>60.5</td>
<td>44.9</td>
<td>20.8</td>
</tr>
<tr>
<td>Hairy cell</td>
<td>2</td>
<td>208-208</td>
<td>2.1-2.3</td>
<td>43.7</td>
<td>54.5</td>
<td>24.6</td>
</tr>
<tr>
<td>Healthy individu</td>
<td>4</td>
<td>176-222</td>
<td>6.0-11.0</td>
<td>46.9</td>
<td>52.4</td>
<td>37.2</td>
</tr>
</tbody>
</table>

* Mean percentage of lysis by sera of all cases studied.

Table 3

Bacteriolytic activity against "autologous" microorganisms

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Microorganism</th>
<th>Source</th>
<th>% of lysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute lymphoblastic leukemia</td>
<td>E. coli, smooth</td>
<td>Urine</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Acute myeloblastic leukemia</td>
<td>E. coli, smooth</td>
<td>Urine</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Acute myelomonocytic leukemia</td>
<td>E. coli, smooth</td>
<td>Urine</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Healthy individuals</td>
<td>Streptococcus</td>
<td>Blood</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

21). Several investigators found impaired phagocytic activity of polymorphonuclears (3, 9, 16, 19, 21). Others, however, were unable to confirm this observation (12, 19, 20). It is surprising, therefore, that the 2nd defense mechanism, namely, humoral antibacterial activity, has not been comprehensively studied in leukemia.

According to present knowledge at least 3 humoral factors are important in the bacteriolytic and bactericidal activity of serum. In the initial phase complement and possibly antibody degrade the lipopolysaccharide-phospholipid complex of the 2 outer layers of the bacterial envelope (5, 6). Lysozyme then penetrates through the "holes" in the envelope and destroys the peptidoglucan polymer of the bacterial wall (5, 6). The microorganism is disrupted subsequently by osmotic forces (6). It seems that antibodies are required in a very minimal amount (if any) and thus probably are not a limiting factor in the antibacterial reactions (8). On the contrary, complement is necessary in the bactericidal process (8, 24) while lysozyme plays an important role in both the lysis and in killing of microorganisms (4, 7).

Not all bacteria are equally susceptible to the action of serum (14). Whereas many gram-negative bacteria were found to be susceptible (14), gram-positive cocci that lack the lipopolysaccharide-phospholipid complex in the envelope are usually resistant (6). It is apparent that investigation of antibacterial humoral activity must be conducted under well-controlled conditions. The sera should be processed in such a way that no decrease in complement or other factors can occur. For detection of minor differences between individual sera, a microorganism that is especially sensitive to the antibacterial action of fresh serum should be used. On the other hand, less susceptible microorganisms should also be used in order to better evaluate the antibacterial potency of the sera tested. Using these criteria we found that sera in mono- and myelomonocytic leukemia with hyperlysozymemia have higher antibacterial activity against E. coli Lilly and other microorganisms than sera of healthy individuals, the most potent being those with normal complement and high lysozyme levels (18). There was no correlation with the concentration of immunoglobulins or transferrin. Since several patients were receiving various chemotherapeutic agents, the antibacterial activity of normal human sera supplemented by various chemotherapeutic agents was evaluated previously (18). In the assays used in the present study, chemotherapeutic agents did not influence the results (18).

Since it was observed that the sera in monocytic and myelomonocytic leukemia have higher antibacterial activity than those of healthy persons, it was important to perform a similar study in other types of leukemia. Such a study was supposed to determine whether various leukemias have different antibacterial activity and whether such activity correlates with any humoral factor.

Our results show that bacteriolytic activity is distinctly different in various types of leukemia, being low in acute lymphoblastic and hairy cell leukemia and higher than normal in chronic myelogenous leukemia. It seems that markedly low serum lysozyme and low bacteriolytic activity in hairy cell leukemia along with the negative staining of hairy cells for myeloperoxidase, nonspecific esterase, and chloroacetate esterase in our patients is in keeping with the presumption that the hairy cells belong to the lymphocytic series.

There was a definite correlation between the bacteriolytic activity and the level of serum lysozyme. Supplementation of serum by a pure human lysozyme corrected even the lowest bacteriolytic activity, as in hairy cell leukemia. In monocytic leukemias with hyperlysozymemia, bacteriolytic activity correlated more with the level of complement than with that of lysozyme (18). However, many of these patients had low levels of complement, and the highest bacteriolytic
activity was observed in those with normal complement and high lysozyme.

In testing bacteriolytic activity against 9 other microorganisms only 3 gram-negative strains were lysed by leukemic sera and no activity against gram-positive microorganisms was detected. It is quite possible that a much longer exposure time or the use of undiluted serum would uncover some antibacterial activity not detected by our assays. In 10 patients bacteriolytic activity against the causative microorganism was tested during acute infection. The sera were unable to lyse such “autologous” bacteria to any reasonable extent at exposure times up to 1 hr.

Quite different results were obtained in testing bactericidal rather than bacteriolytic activity of leukemic sera. Bactericidal activity was higher than normal in acute leukemias and in chronic myelogenous leukemia. Patients with chronic lymphocytic leukemia had normal bactericidal activity. These differences were apparent especially at the lowest dilutions of the sera and a very short exposure time. At higher dilution and longer exposure time bactericidal activity in acute leukemias and chronic myelogenous leukemia was equal to that of the normal sera, and in chronic lymphocytic leukemia it was lower than normal. There was almost no correlation between the bactericidal activity and the level of serum complement, possibly because almost all samples had normal or high complement or because some other unstudied factors were involved in the bactericidal reaction.

It seems that the above-mentioned differences do not fully explain why patients with various types of leukemia have different rates of infection. For the final elucidation of this problem simultaneous investigation of phagocytosis, opsonins, and humoral antibacterial activity should be performed. Such a study is in progress in our laboratory.

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