Inhibiting Effects of Human Plasma and Serum on Neutrophil Random Migration and Chemotaxis

By H. U. Keller, M. W. Hess, and H. Cottier

Various authors have associated increased susceptibility to infectious diseases in certain patients to inhibitors of neutrophil chemotaxis demonstrable in serum or diluted plasma of these patients. The present experiments showed, however, that serum and diluted plasma but not undiluted plasma from normal human donors consistently inhibited chemotactic migration of autologous human neutrophil granulocytes. Therefore, the presence of such inhibitors in the circulating blood can only be assessed by the evaluation of undiluted plasma. The findings suggest that the experimental conditions which have been used in the past to demonstrate such inhibitors in the circulating blood of patients with increased susceptibility to infections are inadequate, and results need reexamination. The inhibitors affect random locomotion and chemotaxis of neutrophil granulocytes but not phagocytosis or the metabolic burst resulting in nitroblue-tetrazolium reduction. On the other hand, phagocytosis of Staphylococcus albus rendered neutrophils chemotactically unresponsive. The significance of so-called cellular defects of neutrophil chemotaxis in such patients is also considered.

CHEMOTACTIC MIGRATION of neutrophil granulocytes to inflammatory sites is part of the host's defence mechanisms against bacterial infections. Defective neutrophil chemotaxis observed in patients with increased susceptibility to infections has been ascribed to three different causes: (1) defects in cytotaxin formation, (2) cellular defects, and/or (3) inhibitors which can be demonstrated in serum or dilute plasma.

We have consistently found inhibitors of neutrophil chemotaxis in serum and dilute plasma of normal human blood donors. Therefore, we thought it useful to reconsider the methods for assessing defects in neutrophil chemotaxis and the interpretation of earlier clinical findings.

MATERIALS AND METHODS

Blood was obtained from 25 normal human donors of the Swiss Red Cross bank. Heparinized blood (10 U/ml) was collected in siliconized tubes. Leukocytes were separated from red cells by the use of Isopaque-methocel, washed with 2% human serum albumin (HSA) in Gey's solution, and resuspended in this medium. Plasma was prepared by centrifugation of heparinized blood and serum from clotted blood. Gey's solution was used for the preparation of diluted serum or plasma (volume/volume). Heparinized (10 U/ml) control media were used in experiments evaluating the effect of plasma. Ovalbumin-rabbit antiovalbumin complexes were prepared at equivalence; washed precipitates were incubated in serum at a concentration of 1 mg/ml serum for 15 min at 37°C and then removed by centrifugation. Leukocytes preincubated with serum or plasma were centrifuged, washed twice with 2% HSA and resuspended in this medium.

Chemotaxis was assessed in conventional Boyden chambers using the two-filter technique, the filters having a pore size of 8 μ (Sartorius Membranfilter GmbH, Göttingen, Germany) and of

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0.45 μ (Millipore, Bedford, U.S.A.), respectively. Chemotactic activity was expressed as the mean number of neutrophils per high power field of triplicate chambers ± standard deviation (SD). Nitroblue tetrazolium (NBT)-tests were performed using heat-killed Candida albicans for stimulation and to assess phagocytosis. Two hundred neutrophils from each sample were evaluated. Heat-killed S. albus washed in 2% HSA in Gey’s solution (2.6 x 10⁶ organisms/ml) was used in phagocytosis experiments. An average of eight organisms/granulocyte was taken up.

RESULTS

The inhibitory action of normal human serum can be demonstrated in two ways: (1) by preincubating the leukocytes in serum before assessing the chemotactic response of the washed cells, and (2) by adding normal serum to the activated serum present in the test solution.

Human neutrophils incubated at 37°C for 30 min with either 50% normal human serum lacking significant chemotactic activity (1.5 ± 0.7 neutrophils/field) or with 50% human serum activated by incubation with immune complexes exhibited decreased random and chemotactic migration as compared to cells pretreated with 2%, HSA (Table 1). Additional experiments of this type showed more clearly that random migration was also decreased following pre-treatment with normal serum. The cell counts obtained per five high power fields were 52 ± 12 neutrophils using cells preincubated in 2%, HSA and 2 ± 2 neutrophils using cells pretreated with 100%, normal serum. The impaired random migration and chemotaxis cannot be explained by a toxic action of the serum during the preincubation period, since this treatment did not lead to significant cell death as judged by total cell counts and/or trypan blue exclusion tests. In addition, preincubation with serum did not impair the capacity of the neutrophils to phagocytose heat-killed C. albicans and to reduce nitroblue-tetrazolium (NBT) following phagocytosis; also, the percentage of neutrophils showing spontaneous NBT-reduction remained unchanged.

Fifty per cent normal human serum exhibiting little if any chemotactic activity also exerted an inhibitory effect on neutrophil chemotaxis when added to 5%, activated serum in the test solution, while addition of activated serum produced no measurable inhibition (Table 2) but rather an increase in chemotactic migration. This indicated that the inhibitory effect of normal serum was, at least under these test conditions, not due to the presence of small amounts of cytotoxins which may not have been detected.

<table>
<thead>
<tr>
<th>Preincubation (30 min at 37°C)</th>
<th>Chemotaxis (Cells/Field ± SD)</th>
<th>Phagocytosis* (C. albicans)</th>
<th>NBT-Reduction† (Spontaneous/Stimulated)</th>
<th>Trypan Blue Exclusion (% + Cells)</th>
<th>Total Cell Counts (cells/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2% HSA</td>
<td>103 ± 18</td>
<td>95</td>
<td>7</td>
<td>97</td>
<td>2 N.D.</td>
</tr>
<tr>
<td>50% normal human serum</td>
<td>1 ± 0.7</td>
<td>95</td>
<td>7</td>
<td>96</td>
<td>3 N.D.</td>
</tr>
<tr>
<td>2% HSA</td>
<td>575 ± 137</td>
<td>96</td>
<td>8</td>
<td>98</td>
<td>6 1.9 x 10⁶</td>
</tr>
<tr>
<td>50% activated human serum</td>
<td>0</td>
<td>96</td>
<td>5</td>
<td>96</td>
<td>5 1.8 x 10⁶</td>
</tr>
</tbody>
</table>

*Per cent neutrophils containing Candida albicans.
†Per cent NBT-positive neutrophils before and after phagocytosis of C. albicans
N.D., not done.
Neutrophils isolated from peripheral blood of normal human donors were capable of showing a good chemotactic response if tested under adequate conditions. This indicated that the inhibitors found in normal serum were not present or operative in the circulating blood. Diluted and later undiluted plasma from normal donors was therefore also evaluated for the presence of inhibitors of neutrophil chemotaxis. Preincubation of leukocytes for 15 min at 37°C with 50% plasma in Gey’s solution resulted in inhibition comparable to that induced by 50% normal serum (Table 3). The inhibitory effect was observed regardless of whether the diluted plasma exhibited chemotactic activity or not. However, undiluted plasma prepared in siliconized glassware had, in contrast to undiluted serum, no significant inhibitory effect (Table 4). Control experiments showed that addition of heparin to serum or 2% HSA used as incubation medium for the cells did not modify their migratory response. This suggests that the inhibitory activity found in normal serum or diluted plasma was generated in vitro. Formation of these factors can also be induced by incubating undiluted plasma in siliconized tubes with immune complexes.

Human neutrophils, like granulocytes from experimental animals,16,17 exhibited impaired migration following phagocytosis of heat-killed and washed S. albus, the values being even lower than random migration in the untreated controls (Table 5). In contrast to controls, a large proportion of the neutrophils which have been exposed to bacteria and which have lost the capacity to express chemotactic responsiveness nonetheless showed intracellular reduction of
Table 4. Effect of Preincubation of Human Leukocytes in 100% Normal Human Serum or Plasma on Neutrophil Chemotaxis

<table>
<thead>
<tr>
<th>Preincubation of Cells (20 min at 37°C)</th>
<th>Cell Suspending Medium</th>
<th>Test Solution</th>
<th>Neutrophils/Field (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2% HSA in Gey’s solution</td>
<td>2% HSA in Gey’s solution</td>
<td>Gey’s solution</td>
<td>35 ± 7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5% human serum + immune complexes</td>
<td>301 ± 22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100% normal human serum</td>
<td>235 ± 19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100% normal human plasma</td>
<td>61 ± 3</td>
</tr>
<tr>
<td>100% human plasma</td>
<td></td>
<td>5% human serum + immune complexes</td>
<td>268 ± 17</td>
</tr>
<tr>
<td>100% human serum</td>
<td></td>
<td>5% human serum + immune complexes</td>
<td>74 ± 20</td>
</tr>
</tbody>
</table>

NBT. Thus, phagocytosis resulted in decreased migration and concomitant stimulation of intracellular NBT reduction.

DISCUSSION

Inhibitors of neutrophil chemotaxis have been detected in sera or dilute plasma of patients and have been related to their increased susceptibility to infectious diseases. Our findings, however, suggest that these data are not representative for in vivo conditions, since such inhibitors were also found in serum and/or diluted plasma of normal blood donors, while they were absent in undiluted plasma prepared with siliconized glassware. Though such inhibitors may in fact be present in patients’ plasma, the methods used hitherto for their detection are inadequate. In some instances, demonstration of the inhibitors is based on experiments comparing the response of neutrophils suspended either in dilute plasma or serum. Formation of inhibitors may thus inadvertently occur in vitro. In addition, cytotaxin formation in the cell suspension and/or the presence of cytotaxins in the circulating blood of patients resulting in a diminished gradient between the two compartments of the chamber has not always been considered. Furthermore, the fact that most authors used dilute solutions—usually 10%—of plasma or serum makes comparisons with in vivo conditions impossible. Thus, the occurrence and the significance of inhibitors of neutrophil chemotaxis in patients still needs to be evaluated.

The inhibitors which we consistently found in normal serum of healthy blood donors appear to have similar characteristics as the ones described by Smith et al. in a child with recurrent infections: they are heat-stable and do not impair viability, phagocytic activity of the granulocytes, and the metabolic burst as expressed by NBT-reduction. Our data demonstrate that these granulocyte functions are to some extent under separate control. But they are not entirely independent, since neutrophils become chemotactically unresponsive following

Table 5. Decreased Migration of Human Neutrophils Following Phagocytosis of Heat-killed S. albus

<table>
<thead>
<tr>
<th>Preincubation of Cells (15 min at 37°C)</th>
<th>Cell Suspending Medium</th>
<th>Test Solution</th>
<th>Neutrophils/Field (Mean ± SD)</th>
<th>% NBT Positive Neutrophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>2% HSA in Gey’s solution</td>
<td>2% HSA in Gey’s solution</td>
<td>Gey’s solution</td>
<td>17 ± 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5% human serum + immune complexes</td>
<td>81 ± 10</td>
<td>4</td>
</tr>
<tr>
<td>2% HSA in Gey’s solution containing S. albus</td>
<td></td>
<td>5% human serum + immune complexes</td>
<td>0 ± 0</td>
<td>78</td>
</tr>
</tbody>
</table>
phagocytosis. It is conceivable that chemotaxis and phagocytosis are at least, to some extent, alternating functions. This hypothesis is compatible with reports on decreased migratory capacity and increased spontaneous NBT reduction in granulocytes of newborns\textsuperscript{19,20} or patients with infectious diseases.\textsuperscript{21,22}

The present findings on serum inhibitors bear also on so-called cellular defects of neutrophil chemotaxis. Neutrophils preincubated with normal serum in vitro show decreased random migration and chemotaxis, while viability, phagocytosis and metabolic burst as expressed by NBT reduction remain unimpaired; i.e., they are comparable to “lazy leukocytes.”\textsuperscript{13} It is reasonable to assume that such inhibitors may become operative in the circulating blood under pathologic conditions in vivo and the neutrophils will then behave like defective cells. Moreover, neutrophils show impaired migration following ingestion of particles in vitro (Table 5). It has been suggested that phagocytosis of immune complexes is responsible for decreased granulocyte emigration in vivo and increased susceptibility to infectious diseases in patients with rheumatoid arthritis.\textsuperscript{18} Thus, the distinction between inhibitors and other factors leading to functional defects of neutrophil migration and intrinsic cellular defects is more difficult than anticipated, and requires a detailed analysis. This is essential to determine if, in a particular patient, defective chemotaxis is the cause and/or the consequence of increased susceptibility to infectious diseases. The present experiments were mainly concerned with the development of adequate techniques for detecting defects in chemotactic migration, in particular decreased migration due to inhibitory factors present in the circulating blood. They showed that chemotactic as well as random migration is markedly reduced following preincubation of cells in serum or diluted plasma. The nature and mode of action of the inhibitory factors need to be further investigated.

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REFERENCES

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