Cell Elastimetry in the Detection of Antineutrophil Antibodies

By Michael E. Miller, Laurence A. Boxer, Eric J. Kawaoka, and Wayne A. Border

Antineutrophil antibodies have been demonstrated in the sera of recipients of multiple transfusions, mothers of infants with transient neonatal neutropenia, and in some patients with idiopathic neutropenia. Although antibodies directed against erythrocytes or platelets have long been recognized as causative mechanisms in the development of, respectively, anemia or thrombocytopenia, a similar cause and effect sequence for antineutrophil antibodies has been questioned. With the development of more sophisticated leuko-agglutinin techniques and functional assays, however, more reliable detection of antineutrophil antibodies has been possible. Consequently, the clinical and laboratory features of patients with autoimmune neutropenia are now better defined.

Although the mechanisms(s) by which antineutrophil antibodies cause neutropenia are unclear, they presumably mediate their effect(s), at least in part, through alteration of the PMN membrane. In order to further explore the possible functional effects of antineutrophil antibodies, we have now applied the technique of cell elastimetry. This is a single cell assay which partially reflects alterations in membrane integrity.

Materials and Methods

Antineutrophil Antibodies

Peripheral blood neutrophils (designated test cells) were obtained as previously described. The cells were incubated with 133 μl of

Randomly coded serums from patients and controls were studied for deformability by observers unaware of the code. (B) In the second group of studies, sera containing immune complexes were incubated with normal PMNs. No significant effects were noted upon deformability. As a single cell assay that partially reflects membrane rigidity, elastimetry may, therefore, have potential in the further characterization of mechanisms by which such antineutrophil antibodies compromise neutrophil functions.

Cell Elastimetry

Elastimetry studies were performed as previously described. In principle, the negative pressure required to aspirate a cell into the orifice of a micropipette was measured. For the study of PMNs, pipettes were drawn from glass capillary tubing with a glass microelectrode puller. After drawing, each pipette tip was examined microscopically for splintered edges and the internal diameter measured using a micrometer eyepiece. Pipettes with rough edges or internal diameters outside the acceptable range (3–5 μ) were discarded.

PMN suspensions were prepared from heparinized, venous blood and suspended to a final concentration of 5 × 10⁶ PMNs/cc medium CMRL 1066 in a 3 mm moist chamber. A PMN was manipulated into the orifice of a suitably mounted micropipette that was connected to a negative pressure source controlled by a sensitive needle valve and read on a mercury column. Negative pressures required to aspirate the entire PMN into the micropipette were then recorded.

Antineutrophil Sera

Eight sera with previously detected antineutrophil antibody activity were studied. Antibodies had been determined by quantitating the initial rate of glucose oxidation by the indicator human PMNs. A test was considered positive if the glucose oxidation rates exceeded two standard deviations above the control mean (n = 14) of 3304 ± 1474 cpm/10⁶ cells/30 min.

Confirmation that the antineutrophil activity found in the serum correlated with the presence of IgG-bound antineutrophil antibodies was obtained by identifying antineutrophil antibodies on donor neutrophils with fluorescein-labeled staphylococcal protein A.

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lymphocytotoxic antibodies and a rabbit antihuman neutrophil serum. Normal PMNs were incubated with a known antineutrophil positive or negative serum at 37°C for 30 min. (All sera had been heat-inactivated at 56°C for 30 min prior to incubation.) Following incubation, the PMNs were tested for deformability (serum still present). In order to ensure objectivity, the study was conducted in an entirely blind fashion. Randomly coded sera from patients and controls were studied for deformability by observers unaware of the code.

Patients

Sera from the following six patients were studied.

Patient 1. A 45-yr-old white female, well until age 45 when she was found to have a pretribial ulcer and splenomegaly. Her blood count revealed a hemoglobin of 11.4, hematocrit of 34, white cell count of 400, with 10% neutrophils. Her platelet count was 280,000. She had a positive direct Coomb's test. Examination of the bone marrow revealed a marked increase in cellularity with an apparent arrest at the myeloid stage of development. She had presence of antineutrophil antibodies prior to steroid treatment with values exceeding 2.7-fold above the control mean and at the nadir of her neutropenia. She responded to therapy with prednisone at 60 mg per day over 2 wk with a return of white cell count to 1800 with 40% neutrophils. After 1 mo of prednisone therapy, her absolute neutrophil count was normal.

Patient 2. A 53-yr-old woman with onset of polyarthritis at age 28, developed neutropenia with a white cell count of 2200 with 25% neutrophils at age 38. She had a splenectomy, and postoperatively, the white cell count ranged from 5600 to 8900 with normal differentials. She was started on prednisone at age 46. Subsequently, she developed a nodular pulmonary abscess and was treated with sulfa drugs along with discontinuation of her steroids at age 49. One year later, she developed osteomyelitis of her right large toe. Her platelet count was 280,000.

Patient 3. A 62-yr-old male with a longstanding history of rheumatoid arthritis, as well as treated tuberculosis, had a positive rheumatoid factor of 1000. He presented with a white cell count of 5700 with an absence of mature peripheral blood neutrophils and bands. Bone marrow revealed normal myeloid activity. The spleen was not palpable; he had antineutrophil activity in his serum 2.3 above the control mean. Muramidase was 30 μg% with normal being up to 20.

Patient 4. A 64-yr-old female, who was receiving diabinese and insulin for diabetes, was found to be neutropenic. Bone marrow revealed a myeloid arrest; white cell count was 1100 with only 8% neutrophils, normal hemoglobin and platelet count. Her serum contained antineutrophil activity that exceeded the control mean by 3.1. She had positive latex fixation at 1/140; she also had polyclonal gammapathy. She was placed on steroids for several months without response and, ultimately, responded to Cytoxan at 125 mg orally for 8 wk with return of a normal white cell count.

Patient 5. A 30-yr-old male with idiopathic neutropenia presented with two episodes of cellulitis and was found to have a white cell count of 1500 with 11% neutrophils and antineutrophil activity in his serum that exceeded the mean by 2.4-fold. His neutrophil count returned to normal after 6 wk of daily prednisone therapy at 60 mg per day.

Patient 6. A 45-yr-old lady with Felty's syndrome presented with a white cell count of 1500 with 16% neutrophils and was treated for 2 mo with prednisone. In spite of prednisone, her white cell count remained at 1200 with 20% neutrophils, and her serum contained antineutrophil activity of 2.6-fold above the control mean.

The determination of the antineutrophil antibodies was performed for the six patients while the patients were neutropenic and prior to initiation of steroid therapy.

Immune Complex Sera

The effects of sera from patients with circulating immune complexes upon PMN deformability were also studied. Six sera were chosen at random from a previously reported group of patients with biopsy-proven glomerulonephritis. Each serum sample was coded so that the performance of elastimetry was carried out by observers unaware of the source of the serum. Immune complexes were measured by the Raji cell radioimmune assay and the radiolabeled C1q binding assay.

Table 1. Patients Studied With Immune Complexes

<table>
<thead>
<tr>
<th>Patient Diagnosis</th>
<th>Raji Assay</th>
<th>C1q Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 1: systemic lupus erythematosus</td>
<td>+ *</td>
<td>+</td>
</tr>
<tr>
<td>No. 2: systemic lupus erythematosus</td>
<td>+</td>
<td>- *</td>
</tr>
<tr>
<td>No. 3: systemic lupus erythematosus</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>No. 4: Sjögren's syndrome</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>No. 5: subacute bacterial endocarditis</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>No. 6: Henoch-Schönlein purpura</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* + = positive; - = negative for circulating immune complexes.

RESULTS

In the first group of studies, normal PMNs were incubated with 1 of 8 known antineutrophil sera or with a known antibody-negative serum. When incubated with normal PMNs, all of the sera with known antineutrophil antibodies effected significantly reduced deformability (Table 2). By contrast, none of the antibody-negative sera showed any effect upon PMN deformability.

Table 2. Deformability of Normal Neutrophils Incubated With Antineutrophil Sera. Measured as Negative Pressure to Completely Aspirate PMNs (cm Hg)

<table>
<thead>
<tr>
<th>Assay</th>
<th>Normal</th>
<th>AIN</th>
<th>AIN</th>
<th>AIN</th>
<th>AIN</th>
<th>ALS</th>
<th>LTab</th>
<th>ANS</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>11.4 ± 1.2 (SEM)</td>
<td>24.2 ± 2.1</td>
<td>&gt;30</td>
<td>23 ± 1.1</td>
<td>27 ± 0.6</td>
<td>26 ± 2.8</td>
<td>24 ± 1.7</td>
<td>&gt;30</td>
</tr>
</tbody>
</table>

Abbreviations: AIN, autoimmune neutropenia; ALS, antilymphocyte sera; LTab, lymphocytotoxic antibodies; ANS, rabbit antihuman neutrophil serum.
Table 3. Deformability of Normal Neutrophils Incubated With Sera From Patients With Known Circulating Immune Complexes. Measured as Negative Pressure to Completely Aspirate PMNs (cm Hg)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Normal (unincubated)</td>
<td>11.4 ± 1.2 (SEM)</td>
</tr>
<tr>
<td>No. 1</td>
<td>10.8 ± 1.8</td>
</tr>
<tr>
<td>No. 2</td>
<td>10.9 ± 1.8</td>
</tr>
<tr>
<td>No. 3</td>
<td>8.7 ± 1.8</td>
</tr>
<tr>
<td>No. 4</td>
<td>9.3 ± 1.5</td>
</tr>
<tr>
<td>No. 5</td>
<td>8.6 ± 1.6</td>
</tr>
<tr>
<td>No. 6</td>
<td>8.9 ± 1.7</td>
</tr>
</tbody>
</table>

See text for details of patients.

normal PMNs. No significant effects were noted upon deformability (Table 3).

DISCUSSION

The clinical and laboratory consequences of anti-neutrophil antibodies have recently been better appreciated. The precise mechanism(s) by which such antibodies produce neutropenia are unclear, but it is likely that some degree of membrane damage or alteration occurs.\(^1\)\(^5\) Thus, it has been possible to detect such antibodies by a variety of assays that reflect membrane involvement.

In this study, eight sera with known antineutrophil activity were incubated with normal PMNs, and each effected a significant reduction in deformability. Two of the eight sera (rabbit antilymphocyte sera and that from a multiply-transfused patient) were not fully absorbed prior to testing and, therefore, contained antineutrophil activities in addition to their known antilymphocyte activities.

Although the antineutrophil antisera appeared directly to affect cell elasticity, it remained possible that immune complexes present in serums of the antineutrophil antibody-positive individuals might result in secondary damage or compromise to the neutrophil membrane. If true, this would argue against the direct antineutrophil effect we have postulated. To explore this possibility, sera from patients with circulating immune complexes were incubated with normal PMNs. No effects upon deformability were noted.

While a number of factors may determine the deformability of a cell, surface rigidity is one of the major determinants.\(^6\)\(^9\) Our data show that PMNs incubated with antineutrophil antibodies become less deformable. Elastimetry is a single cell assay. As such, it may have potential in the further characterization of mechanisms by which such antibodies compromise neutrophil functions.

ACKNOWLEDGMENT

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REFERENCES

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