Protein A Adsorption of Acute Myelogenous Leukemia Serum Induces In Vitro Blast Lysis

By Wesley J. Miller, Richard F. Branda, David D. Hurd, William Wachsman, Nancy L. Nelson, and Harry S. Jacob

We studied cytotoxic activity of acute myelogenous leukemia (AML) sera for AML blasts before and after immunoadsorption with *Staphylococcus aureus*, Cowan I (SAC), which contains protein A. We found in vitro that incubation with treated AML sera reduced viability to 42.7% of control (p < 0.01) for autologous and 21.0% of control (p < 0.01) for allogeneic blasts. Normal peripheral blood cells were not killed by treated AML sera. Wood 46 strain of *Staphylococcus aureus*, which does not contain protein A, did not significantly reduce AML blast viability (94.8%, p > 0.4), while Sepharose-bound protein A reduced viability to 63.8% (p < 0.01). Cytotoxicity does not appear to be complement-mediated, but cytotoxic activity is trypsin-sensitive and is contained in the immunoglobulin fraction. This model for study of the tumoricidal action of protein A adsorption should be useful for predicting utility of plasma adsorption as a therapeutic adjunct in the future.

**MATERIALS AND METHODS**

Peripheral blood blasts and sera were obtained from patients with AML prior to the initiation of chemotherapy. Blast populations were enriched using Ficoll-Hypaque interface mononuclear cells. Sera were adsorbed using *Staphylococcus aureus* Cowan I (SAC) as a 10% suspension (Pansorbin, Calbiochem, La Jolla, Calif.)*Staphylococcus aureus* Wood 46 strain (10% suspension, Sansorbin, Calbiochem), or purified protein A bound to Sepharose 4B beads (Pharmacia, Uppsala, Sweden). Sera were adsorbed for 15 min at room temperature using particulate SAC or Wood 46 (washed and centrifuged to remove preservatives) from 1 ml of the 10% suspension, or 30 mg Sepharose protein A, per milliliter of serum. After incubation sera were centrifuged and passed through a 0.22-μ filter to remove the adsorbent particles, and then diluted to 15% with CMRL 1066 culture media containing glutamine and penicillin-streptomycin. Target cells, (5 x 10^6) were incubated in 1 ml of the media-serum mixture at 37°C, 5% CO₂ for 24 hr and were then counted by Coulter Counter and viabilities determined by Trypan blue exclusion.

The immunoglobulin fraction of AML serum was purified using a DEAE-sephadex batch technique, dialyzed, and diluted to 790 mg/dl in culture media for addition to culture.

The blast target cell population was further purified in certain experiments using adherence to remove monocytes and sheep red cell rosetting combined with Ficoll-Hypaque centrifugation to remove T cells.

Trypsinization of serum samples was accomplished using polyacrylamide-bound insoluble Trypsin (Sigma [St. Louis, Mo.], activity 235 U/g). Half-milliliter samples of serum were incubated with 10 mg of insoluble Trypsin at 30°C, pH 7.6, for 1 hr, followed by centrifugation and filtration to remove the Trypsin.

**RESULTS**

The results of cultures with variously treated sera are shown in Table 1. All results are expressed as viable cells compared to control incubations (the same cells incubated with untreated serum). Incubation of AML blasts from 26 patients with autologous SAC-treated sera resulted in a highly significant reduction in viability to 42.7% of control (p < 0.01 by paired t test). However, there was a marked variability, with some cultures showing virtually no viable cells while others showed no reduction in viability. Adsorption of AML sera against Wood 46 strain (which does not bear protein A) resulted in 94.8% of control viability.

**REVIEWERS**

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incubated with only 1.5% SAC-treated AML serum. Purified protein A bound to Sepharose beads (Sepharose PA). Staphylococcus aureus untreated AML sera. Immunoadsorbents include protein-A-bearing substances that either support these data suggest that untreated AML sera contain serum) significantly raised viability or untreated sera did not similarly led to no reduction in viability when blasts were incubated in treated sera. In addition of 0.15 ml of untreated autologous serum to media and I control cultures. Normal autologous serum. Allogeneic AML sera specifically reduce blast viability (93.2% ± 19.0%, p < 0.01, n = 26). Viabilities in experimental cultures are expressed as a percentage of viable cells in 24-hr culture with treated serum compared to control incubations with untreated AML sera. Immunoadsorbents include protein-A-bearing Staphylococcus aureus Cowan I (SAC); non-protein-A-bearing S. aureus Wood 46; purified protein A bound to Sepharose beads (Sepharose PA).

Ability of untreated serum to interfere with cytotoxicity was studied utilizing mixing experiments as shown in Table 1. In 7 cultures, SAC-treated sera alone reduced viability to 30.1% of control, whereas addition of 0.15 ml of untreated autologous serum to parallel cultures (also containing 0.15 ml of treated serum) significantly raised viability to 77.6% (p < 0.001 by paired t test). Adding 0.15 ml of media or treated sera did not similarly improve viability. Additionally, SAC-adsorption of media (without serum) led to no reduction in viability when blasts were incubated in treated media and 15% untreated serum. These data suggest that untreated AML sera contain substances that either support viability or block cytotoxicity, and that such factors can be removed or inactivated by SAC adsorption. Direct contact of serum with SAC seems to be essential for induction of this effect.

The specificity of the SAC effect was studied utilizing allogeneic sera. In 7 cases, incubation with SAC-treated allogeneic AML sera led to a marked reduction in viable cells (21.0% compared to control, p < 0.01), whereas 5 serum samples from normals treated with SAC caused no significant reduction in AML blast viability (93.2% ± 19.0%, p > 0.4) (Table 1). In 12 experiments, normal peripheral blood mononuclear cells remained viable when incubated with SAC-adsorbed AML (97.8% ± 19.8%) or SAC-adsorbed normal autologous sera (86.8% ± 13.5%). These experiments suggest that SAC-treated AML sera specifically reduce AML blast viability in short-term culture.

Mechanisms by which SAC adsorption might cause cytotoxicity were also considered. Specifically, the possibilities that the cytotoxic effect required complement, immune effector cells, or serum immunoglobulins were examined. Seven AML sera were heat-inactivated at 56°C for 30 min to inactivate complement prior to immunoadsorption: a similar reduction in viability was seen with (39.6% mean viability) or without (40.7% viability) heat-inactivation of sera followed by SAC adsorption. Since Ficoll-Hypaque-separated cells are not pure blast populations, we also entertained the possibility of cell-mediated or antibody-dependent cell-mediated cytotoxicity (ADCC) brought about by the few remaining nonblast mononuclear cells. However, removal of the adherent cell population (mainly monocytes) from 4 samples

### Table 1. Adsorbed AML Sera Specifically Lyse AML Blasts

<table>
<thead>
<tr>
<th>Serum</th>
<th>Treatment</th>
<th>AML Cells (%)</th>
<th>Normal Cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML†</td>
<td>SAC</td>
<td>42.7 ± 30.1</td>
<td>ND</td>
</tr>
<tr>
<td>AML</td>
<td>Wood 46</td>
<td>94.8 ± 25.9</td>
<td>ND</td>
</tr>
<tr>
<td>AML</td>
<td>Sepharose PA</td>
<td>63.8 ± 28.4</td>
<td>ND</td>
</tr>
<tr>
<td>AML</td>
<td>SAC + Untreated</td>
<td>77.6 ± 24.0</td>
<td>(p &lt; 0.001, n = 7)</td>
</tr>
<tr>
<td>Allogeneic AML</td>
<td>SAC</td>
<td>30.1 ± 25.1</td>
<td>97.8 ± 19.8</td>
</tr>
<tr>
<td>Normal AML</td>
<td>SAC</td>
<td>93.2 ± 19.0</td>
<td>86.8 ± 13.4</td>
</tr>
</tbody>
</table>

*p Value

†Autologous AML incubations except where specified.

§Mixing experiments were done using equal volumes of SAC-treated and untreated autologous AML serum. (SAC) represents the same samples incubated with only 15% SAC-treated AML serum. *p Value for this set of experiments was derived by paired t testing comparing these two groups.

Normal autologous serum.
resulted in no significant increase in viability of cells cultured with treated sera (49.7% compared to 38.3% without removal of adherent cells). Furthermore, removal of T lymphocytes by sheep red cell rosetting of target cells also did not improve viability (32.1% viability in culture with SAC-treated AML serum). Thus, in limited trials, blast death induced by SAC-treated serum does not appear to be complement-mediated, nor is it altered by removal of cells by adherence or sheep red cell rosetting.

To explore the role of immunoglobulin in cytotoxicity, we chromatographically purified the immunoglobulin fraction of AML sera. This fraction supported blast viability when added to culture (0.15 ml of fraction at 790 mg/dl in media containing no serum); however, when preadsorbed with SAC (which decreased immunoglobulin levels to 390 mg/dl), cell viability was markedly reduced (26.1% of control viability, n = 3). Thus, the immunoglobulin fraction appears to contain a cytotoxic component that is unmasked by SAC adsorption, or alternatively, some part of the immunoglobulin fraction is necessary for the support of cell viability in these cultures. Further confirmation that a serum protein is mediating cytotoxicity in these cultures was obtained utilizing Trypsin. In four cultures, SAC-treated sera reduced viability to 59.2% ± 29.2%. When these sera were trypsinized following SAC treatment and incubated with the same autologous blast populations (simultaneous cultures), the viability was 103.2% ± 30.1% compared to control cultures. Parallel cultures with sera trypsinized but not SAC-treated led to 99.8% ± 15.1% viability. Although preliminary, these data suggest that the SAC effect is mediated by a protein and that the effect is completely eliminated by enzymatic destruction of protein.

To further explore the role of immunoglobulin, multiple adsorptions were performed on 5 samples to remove increasing amounts of IgG. Maximal cell death was noted after one or two adsorptions (viability 60.4% of control); further adsorption caused improved viability, and after 5 adsorptions sera supported viability completely (91.2 ± 12.3%). These findings suggest that the initial adsorptions allow the expression of cytotoxicity (e.g., by removal of blocking substances), but further adsorptions actually remove or inactivate the cytotoxic substance (e.g., a cytotoxic immunoglobulin). Furthermore, the effect of protein A columns is saturable. That is, an aliquot of serum passed over protein A develops cytotoxic activity toward autologous blast cells. A second aliquot of serum passed over the same protein A acts like untreated control serum in culture.

**DISCUSSION**

Our studies demonstrate that most AML sera can be rendered specifically cytotoxic for AML blasts by protein A adsorption. We believe that this specific blast death is due to an effect of protein A on leukemic serum, rather than a nonspecific effect since: (1) protein-A-treated normal serum is not toxic to leukemia myeloblasts nor to normal peripheral blood mononuclear cells; (2) protein A treatment of media (without sera) from our cultures results in no reduction in cell viability; (3) Wood-46-treated AML serum is nontoxic; (4) both purified protein A bound to Sepharose and protein-A-bearing staphylococcal cell wall are effective inducers of cytotoxicity; and (5) addition of fresh serum to protein-A-treated serum enhances blast viability.

Although the exact mechanism of this specific protein-A-induced cytotoxicity is unclear, our studies suggest that it is protein-mediated (probably immunoglobulin), does not require complement, and utilizes few, if any, effector cells. SAC may selectively remove a "blocking" antibody or immune complex, leaving a cytotoxic antibody, possibly of the IgG3 subclass. Alternatively, SAC might remove or alter the effect of a necessary growth or viability factor found in AML sera. Finally, we cannot exclude the possibility that small amounts of protein A (possibly complexed to immunoglobulin) are released into culture and mediate cytotoxicity. At present, we favor the first explanation. The clarification of mechanism(s) awaits further studies, but the described culture system should be useful in the future for predicting therapeutic utility of protein A adsorption by extracorporeal perfusion. In this regard, animal studies (reported preliminarily elsewhere) indicate that this may be the case.

**REFERENCES**


Protein A adsorption of acute myelogenous leukemia serum induces in vitro blast lysis

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