Biological and Immunological Characterization of ATG and ALG

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Antithymocyte globulin (ATG) and antilymphocyte globulin (ALG) are effective therapies in aplastic anemia: their mechanism of action is undefined. We assayed multiple properties of ATG and ALG to address the biological and immunological bases for differences between ATG and ALG and lot variation. In addition, we studied a lot reported to be inactive in an American clinical trial; however, in retrospect, this lot appeared to be active in patients treated in Europe. Immunoprecipitation of thymocyte and lymphocyte membrane proteins with ATG and ALG showed binding of a common monoclonal antibody was assessed using flow cytometry and a radiolabeled assay. In general, there was less lot variation among ALGs than ATGs; however, all ALG lots were more potent blockers of binding of anti-HLA-DR and anti-Leu 1 antibodies than was ATG. Both ALG and ATG effectively blocked binding of anti-Leu 2a, anti-Leu 3a, anti-Leu 4, anti-Leu 5b, and anti-IL 2 receptor abs; neither blocked binding of anti-Leu 7. All preparations were capable of inducing T-cell blastogenesis, although there were considerable lot variation. All lots lysed 60% to 75% T cells in a rabbit complement-mediated cytotoxicity assay, with most having a plateau of activity at 5 to 10 ug/mL. Two lots of ALG, including the lot reported to be clinically inactive, showed less toxicity at suboptimal concentrations and did not plateau even at 80 ug/mL. In total, these results indicate important differences between ATG and ALG in general, more lot variation among ALGs than ATGs and only differences in cytotoxicity between an “inactive” lot of ALG and most, but not all, other active ATG and ALG preparations.

MATERIALS AND METHODS

ATG and ALG preparations. ATG lots 17924, 844, and 731 were provided by the Upjohn Company (Kalamazoo, Mich) and ALG lots 160, 162, 15343, 181 S, and 97412 by the Swiss Serum and Vaccine Institute (Berne, Switzerland) as sterile solutions at 50 mg/mL in saline. The protein concentration confirmed spectrophotometrically and the preparations determined to be greater than 95% IgG by SDS-polyacrylamide gel electrophoresis (PAGE) and Coomassie blue staining.

ATG was prepared by injecting horses with homogenized emulsions of normal thymocytes removed from children undergoing cardiac surgery. Antibodies to RBC stroma and human plasma proteins were removed by absorption before the gamma globulin was purified. All ATG lots represent pooled sera from four inoculated animals, obtained and processed at different times. ALG lot 17924 has been successfully employed in clinical trials with response rates to transfusion independence in 40% to 80% of patients. The discovery of the usefulness of ALG was based empirically on clinical studies and not dependent on knowledge of the precise etiology of bone marrow failure. A likely mechanism of action of ATG and ALG is immunosuppression, as some proportion of cases of aplastic anemia appear to be immune mediated; however, ATG also has been shown to stimulate T-cell proliferation and lymphokine production, and a direct effect on hematopoietic progenitors has been inferred by some investigators. Without an established mechanism of action for ATG and ALG, prospective testing of horse sera for clinical activity in aplastic anemia has not had a firm rational basis.

Despite their effectiveness, there are intrinsic difficulties in using animal sera as treatment, including the broad specificity of these antisera for human cells, allergic reactions like serum sickness, important differences in their methods of production, uncertain formulations, and potential lot variation. In fact, one batch of ALG (lot 97412) has been reported to be clinically ineffective, raising the issue of significant lot variation. Because of the practical and theoretical uncertainties concerning the constitution of ALG and ATG, we undertook to characterize in detail this lot and other preparations using a variety of biological and immunological assays. Special emphasis was placed on differences between ATG and ALG in general, lot variation within each type of horse serum preparation, and laboratory correlates of clinical activity.
density sedimentation, washed three times, and resuspended in appropriate media. T cells were fractionated by rosetting with neuraminidase treated sheep red blood cells overnight at 4°C. Phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PHA-PBMC) were obtained by incubating PBMC in RPMI 1640 supplemented with 2 mmol/L glutamine, 20% fetal calf serum (Hyclone, Logan, Utah), and 1% penicillin and streptomycin (Grand Island, NY) at a final concentration of 10 ug/mL neuraminidase treated sheep red blood cells. After 48 hours, the cells were washed, resuspended in the same media without PHA but supplemented with 10% partially purified T-cell growth factor (Cellular Products, Portland, ME), and incubated for an additional 48 to 72 hours before harvesting. Thymocytes were obtained from a child undergoing cardiac surgery, frozen in media containing 10% dimethylsulfoxide, and stored in a liquid nitrogen freezer until used.

**Cytotoxicity assays.** T cells were labeled with 200 μL of 51Cr (1 mCi/mL solution, Amersham, Arlington Heights, Ill) per 1 x 10⁶ cells for one hour at 37°C, washed, and resuspended in RPMI plus 0.1% BSA at a concentration of 20,000 cpm/75 μL. Seventy-five μL of freshly prepared dilutions of heat inactivated ATG, ALG, or nonimmune horse IgG (HlgG, United States Biochemical Corp., Cleveland, OH) was added to 75 μL of labeled cells in triplicate and the cells were incubated for 30 minutes at room temperature. Rabbit complement (Cedarlane, Hornsby, Ontario) at an optimal final dilution of 1:6 or human anti-AB antibody negative serum at a final concentration of 1:24 was then added and the incubation was continued for an additional 60 minutes at 37°C. The cells were then centrifuged and cytocentrifuged with FACS (FACS, Becton Dickinson, Sunnyvale, Calif) and analyzed by flow microfluorometry using an Epics V (Coulter, Hialeah, Fla). Fluorescence was measured using a logarithmic scale. Using the IMMUNO program of the EASY 88 Computer System (Coulter), negative cells in the control samples were subtracted from the samples preincubated with ATG or ALG. This program therefore effects a decrease in mean fluorescence proportional to the blocking of the cell surface protein by the preincubation preparation, but does not alter the proportion of cells scored as positive by comparison to control (nonimmune mouse monoclonal antibody) preparations. Extrapolation from these data allowed an estimation of the amount of the preincubation preparation needed for 50% blocking of each FITC-Ab. In most cases, preincubation with the highest concentration of HlgG resulted in less than 5% blocking; in the occasional case in which this nonspecific blocking was greater than 5%, the mean fluorescence of the sample preincubated with HlgG was used as the control.

**Blastogenesis assay.** The ability of ATG, ALG to stimulate blastogenesis of PBMC was assayed by measuring DNA synthesis by methyl 3H-thymidine incorporation as previously described. The stimulatory index (SI) was computed as the ratio of induced to spontaneous isotope incorporation. In 2 experiments, the proliferating cells were determined by analyzing by flow microfluorometry the proportion of activated T cells (Leu 4, IL 2 receptor+) and B cells (Leu 12) before and after stimulation.

**Flow microfluorometry analysis.** For blocking experiments, PBMC or PHA-PBMC were washed and resuspended in Hanks balanced salt solution (HBSS; M.A. Bioproducts, Walkersville, Md) supplemented with 0.1% bovine serum albumin (BSA) and 0.1% sodium azide (NaNO₃). 1 x 10⁶ cells were preincubated with 100 μL of HlgG, ATG, ALG, or media in varying concentrations for 30 minutes at 4°C, washed twice, and then incubated with 5 μL of commercially available monoclonal antibodies or control IgG directly conjugated to fluorescein (FITC-Ab, Becton Dickinson, Sunnyvale, Calif) at an additional 30 minutes at 4°C in the dark. The cells were washed twice, resuspended in HBSS plus 0.1% BSA and 0.1% NaN₃, and analyzed by flow microfluorometry using an Epics V (Coulter, Hialeah, Fla). Fluorescence was measured using a logarithmic scale. Using the IMMUNO program of the EASY 88 Computer System (Coulter), negative cells in the control samples (ie, samples preincubated with media alone before labeled with FITC-Ab) were subtracted from the corresponding fluorescent channel from the samples preincubated with ATG or ALG. This program therefore effects a decrease in mean fluorescence proportional to the blocking of the cell surface protein by the preincubation preparation, but does not alter the proportion of cells scored as positive by comparison to control (nonimmune mouse monoclonal antibody) preparations. Extrapolation from these data allowed an estimation of the amount of the preincubation preparation needed for 50% blocking of each FITC-Ab. In most cases, preincubation with the highest concentration of HlgG resulted in less than 5% blocking; in the occasional case in which this nonspecific blocking was greater than 5%, the mean fluorescence of the sample preincubated with HlgG was used as the control.

**3H-anti-Tac blocking studies.** The ability of ATG, ALG, and HlgG to block a 3H-monoclonal antibody directed against the interleukin-2 (IL 2) receptor (3H-anti-Tac) from binding to HUT 102 cells (which contain approximately 300,000 IL-2 receptors per cell) was determined using a modification of a previously described assay. ¹⁰⁶ HUT 102 cells were washed in PBS and resuspended in binding media as previously described. Cells were incubated with varying dilutions of unlabeled anti-Tac, HlgG, ATG, or ALG for two hours at room temperature in duplicate. 5.5 ng of 3H-anti-Tac (equivalent to 15,000 cpm, kindly supplied by Dr Thomas Waldmann) was added and the cells incubated for an additional 60 minutes at room temperature. Cell bound radioactivity was determined after the reaction mixture was centrifuged through a 1 mol/L sucrose cushion and the supernatant aspirated. Unbound 3H-anti-Tac was determined by subtracting antibody bound from total antibody added.
Cell surface iodination, immunoprecipitation, and electrophoresis. 10^6 cells were washed and resuspended in Dulbecco's phosphate-buffered saline without calcium or magnesium (DPBS; GIBCO) and the cell surface proteins radiolabeled with 2 mCi of ^125I-sodium iodide (New England Nuclear, Boston, Mass) via the lactoperoxidase-H_2O_2 method. Cells were washed 3 times in DPBS containing 1 mmol/L phenylmethylsulfonylfluoride (PMSF) and 1 mmol/L diisopropylflourophosphate (DFP, both from Sigma, St. Louis, Mo) and the iodinated proteins solubilized by lysing the cells in 1 mL of DPBS containing 1 mmol/L PMSF, 1 mmol/L DFP, and 0.5% NP-40 for 30 minutes at 4°C followed by centrifugation to remove cellular debris. To immunoprecipitate cell surface proteins with ATG and ALG, 50 μL of solubilized iodinated lysate from PHA-PBMNC or thymocytes were precleared twice with 300 μL of 10% Staph protein A (Immunoprecipitin, Bethesda Research Labs, Bethesda, Md) and then incubated with 20 μL of antibody lots at a concentration of 50 mg/mL overnight at 4°C. Bound lysate was immunoprecipitated with Staph protein A, washed 3 times in DPBS containing 0.1% BSA, 0.02% sodium azide, 0.5% NP-40, and 0.01% sodium dodecyl sulfate (SDS, Bio-Rad Laboratories, Richmond, Calif), and resuspended in a reducing sample buffer according to Laemmli. In preliminary experiments, comparable results were obtained using Staph protein A and rabbit antihorse immunoglobulin coated Staph protein A. Final pellets were boiled, Staph protein A removed by centrifugation, and the samples subjected to SDS-polyacrylamide gel electrophoresis using 5% to 15% discontinuous linear gradient gels. After electrophoresis, the gels were fixed, and fluorographed for 1 to 10 days at ~70°C using intensifier screens and Kodak X-O-Mat film.

RESULTS

Immunoprecipitation of cell surface membrane proteins. To determine which cell surface proteins were immunoprecipitated by ATG and ALG, cells similar to the immunogens used for the preparation of ATG and ALG were employed. Both ATG and ALG precipitated a discrete number of major protein bands from membranes of both PHA-PBMNC (Fig 1) and thymocytes (data not shown), without apparent major differences in results using the two cell types. Both ATG and ALG preparations consistently precipitated several major protein components, for example, proteins of molecular weight 182,000, 138,000, 112,000, 93,000, 43,000, and 35,000. However, other protein components were better or exclusively precipitated by one or the other preparation: for example, ATG uniquely precipitated bands of 295,000 and 30,000 and ALG a band of 166,000. Two lots of ATG (844 and 17924) had identical patterns of immunoprecipitation of thymocyte and PHA-PBMNC surface proteins. In contrast, there was variability in the number and intensity of proteins immunoprecipitated by different ALG preparations (lots EC162, 15343, and 97412).

Definition of antibody specificities. In order to define specific antibody specificities in horse sera, the binding of fluoresceinated monoclonal antibodies to T-cell antigens was quantitated after preincubation of cells with ATG or ALG. A typical experiment, illustrated in Fig 2a, shows that increasing concentrations of ATG 844 progressively inhibited binding of anti-Leu 4-FITC as demonstrated by a decrease in mean fluorescence. The blocking ability of different lots of ATG and ALG for anti-Leu 4-FITC varied (Fig 2b), presumably reflecting differences in the concentration of this antibody specificity within these preparations.

Extrapolation from these data allowed an estimation of the concentration of ATG, ALG, or unlabeled monoclonal antibody needed for a 50% decrease in mean fluorescence (Table 2). Comparison of the relative blocking ability for the various antibodies is complicated by differences in antigen quantity on the cell surface, unknown antibody affinities for antigens, and the likelihood of significant interactions among antibodies of differing specificity. However, these results do allow a qualitative comparison of different preparations of ATG and ALG for each monoclonal antibody. Comparison of an ATG lot (844) and an ALG lot (EC162) showed similar amounts of blocking ability for antigens recognized by monoclonal antibodies to Leu 2a, Leu 3a, Leu 4, and Leu 5b. However, ALG EC162 was over 30 times more potent than ATG 844 in blocking anti-Leu 1 (50% inhibition concentration: 5.8 mg/mL vs. 0.16 mg/mL) and more than 20 times more potent in blocking anti-HLA-DR binding (50% inhibition concentration: 104 mg/mL vs. 4.7 mg/mL).

These differences in the blocking ability of ATG and ALG were not due to lot variation. The 50% blocking concentrations for ATG lots 17924 and 844 were similar, with less than twofold variation for all monoclonal antibodies tested (data not shown). Parallel to the immunoprecipitation data, however, the differences among different ALG lots were more pronounced. ALG EC162 was more potent than ALG 97412 in blocking antibody binding to Leu 1 (4 x), Leu 2a (3 x), Leu 4 (4 x), and Leu 5b (3 x); these preparations were similar in their ability to block binding of anti-Leu 3a and anti-HLA-DR. In general, lot 15343 was more similar to 97412 than to EC162. However, even the least potent ALG lot was still 10 times more potent than ATG in preventing the binding of anti-Leu 1 and anti-HLA-DR antibodies.

This analysis was not able to demonstrate antibodies in ATG or ALG able to block the binding of monoclonal
antibodies to Leu 7 (a marker for large granular lymphocytes) or the interleukin 2 receptor (Table 2). However, as both thymocytes and thoracic duct lymphocytes include cells that express the interleukin-2 receptor, 21,22 a more sensitive assay evaluating the ability of these horse immunoglobulin preparations to block the binding of a tritiated monoclonal antibody reactive with the interleukin-2 receptor (called anti-Tac) to HUT 102 cells was performed (Fig 3). Under these conditions, both ATG and ALG were able to significantly block the binding of $^3$H-anti-Tac with equal potency. The potency of ATGs and ALGs compared to the specific antibody reactive with the interleukin-2 receptor, $^2_{22}$ a more sensitive assay than medium alone (control, upper panel) or various lots of ATG or ALG at the same concentration. The decrease in mean fluorescence (MF) is proportional to the relative blocking ability of the different preparations.

**Table 2. Comparison of Antibody Specificities in ATG and ALG**

<table>
<thead>
<tr>
<th>FITC-Ab</th>
<th>WHO &quot;CD&quot; Classification†</th>
<th>Unlabeled Antibody</th>
<th>ATG 844</th>
<th>ALG EC162</th>
<th>ALG 97412</th>
<th>ALG 15343</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Leu 1 (pan-T cell antigen)</td>
<td>CD5</td>
<td>0.0001</td>
<td>5.8 ± 1.1</td>
<td>0.16 ± 0.06</td>
<td>0.61 ± 0.18</td>
<td>0.71 ± 0.25</td>
</tr>
<tr>
<td>Anti-Leu 2a (suppressor/cytotoxic T cells)</td>
<td>CD8</td>
<td>0.0020</td>
<td>0.83 ± 0.34</td>
<td>0.50 ± 0.33</td>
<td>1.5 ± 0.8</td>
<td>0.98 ± 0.40</td>
</tr>
<tr>
<td>Anti-Leu 3a (helper T Cells)</td>
<td>CD4</td>
<td>0.0001</td>
<td>1.6 ± 0.7</td>
<td>1.5 ± 0.8</td>
<td>3.9 ± 1.7</td>
<td>4.2 ± 0.5</td>
</tr>
<tr>
<td>Anti-Leu 4 (T-cell receptor-associated antigen)</td>
<td>CD3</td>
<td>0.0010</td>
<td>3.8 ± 1.5</td>
<td>2.9 ± 0.9</td>
<td>10.3 ± 3.0</td>
<td>9.7 ± 6.6</td>
</tr>
<tr>
<td>Anti-Leu 5b (E-rosette receptor)</td>
<td>CD2</td>
<td>0.0001</td>
<td>0.24 ± 0.06</td>
<td>0.18 ± 0.06</td>
<td>0.49 ± 0.04</td>
<td>0.48 ± 0.02</td>
</tr>
<tr>
<td>Anti-leu 7 (LGL, NK cells)</td>
<td>—</td>
<td>0.0400</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>NT</td>
</tr>
<tr>
<td>Anti-HLA-DR (activated T cells)</td>
<td>DR</td>
<td>0.0100</td>
<td>104 ± 77</td>
<td>4.7 ± 2.5</td>
<td>7.8 ± 5.8</td>
<td>10.0 ± 8.6</td>
</tr>
<tr>
<td>Anti-IL 2 Receptor</td>
<td>CD25</td>
<td>0.0003</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>NT</td>
</tr>
</tbody>
</table>

WHO, World Health Organization; LGL, large granular lymphocytes; NK, natural killer; NT, not tested.

*Less than 10% blocking with the highest dilution (50 mg/mL) of ATG/ALG.

†Values represent the mean ± SD of the extrapolated concentration of ATG, ALG, or unlabeled antibody in 2 to 6 determinations that resulted in a 50% decrease in FITC-Ab mean fluorescence.

‡Cluster designation of antigens as defined by the First and Second International Workshop on Human Leucocyte Differentiation Antigens (reference 24 and 25).
genesis was often inhibited, even though cells were still viable. This effect may have been due to the blocking of the interleukin-2 receptor at these concentrations.

**Cytotoxicity.** All preparations of ATG and ALG tested were effective in a complement-dependent cytotoxicity assay using T cells as targets and rabbit serum as a source of complement (Fig 4). All lots of ATG and ALG had a peak cytotoxicity of 60% to 75%, with most having a plateau concentration between 5.0 to 10.0 ug/mL; significant basis was not demonstrable at concentrations below 0.5 ug/mL. Although ALGs 97412 and 181S had peak cytotoxicity values comparable to other lots tested, they differed in that a plateau was never reached over the range of concentrations tested. At suboptimal concentrations (2.5 to 10 ug/mL), these same lots were significantly less cytotoxic than the other ATG and ALG preparations tested. Similar results were obtained using human serum as a source of complement, except 10 to 15 times higher concentrations of horse sera were needed for similar degrees of cytotoxicity (data not shown).

**Discussion**

Both ATG and ALG precipitated a limited number of antigens from the cell surface membranes of thymocytes and lymphocytes. These bands almost certainly do not reflect all the antigens recognized by the horse sera, as the presence of certain major protein components will obscure minor bands during exposure of the autoradiograph. In addition, some of the densest bands precipitated most likely represent common cell membrane proteins, as ATG has been shown to recognize many normal human cell types and their molecular weights do not correspond to those for known T-cell-specific proteins. Nonetheless, while the patterns of immunoprecipitation were roughly similar within ATG and ALG lots, differences in proteins precipitated by ATG compared to ALG were evident. These differences were also recognized in quantitative blocking experiments with monoclonal antibodies to T-cell antigens. Remarkable in these experiments was the much greater ability of ALG to block binding of at least two antigens, the pan-T cell antigen Leu 1 and HLA-DR. While there was greater variability among lots of ALG than among lots of ATG in this assay, even the least potent lot of ALG was 10 times more active in blocking binding to these
antigens. That the blocking ability of ATG and ALG was demonstrated in a case in which we were technically able to immunoprecipitate with monoclonal antibodies; in this instance, preclearing of lysates with ATG and ALG but not HlgG removed HLA-DR antigens (data not shown).

All the ATG and ALG preparations tested showed cytotoxic activity for T cells. Despite their poorer performance in some antibody competitions, all the ATG lots were equivalent to the best ALG lots in cytotoxicity. The ability to effect lysis must depend not only on the specific antigens recognized, but on factors such as the physical arrangement of antigens on the cell surface and the ability of antibodies to fix complement. There were no relative differences in cytotoxicity using rabbit or human serum as a source of complement. Furthermore, the concentrations of horse antisera needed for maximal cytotoxicity with human sera (160 to 320 ìg/mL) were lower than the reported mean peak plasma levels of equine IgG after ATG infusion (727 ± 310 ìg/mL).96,31 It may be difficult to relate in vitro cytotoxicity experiments to clinical performance as immunosuppressive agents, as the correspondence might be, for example, to suboptimal as opposed to plateau concentrations measured in vitro or dependent on the regimen for administration of sera to patients. The in vitro data suggests that the in vivo properties of ATG and ALG may be concentration related; T cells may be stimulated at low concentrations, whereas at higher concentrations, complement-mediated cytotoxicity and inhibition of T-cell proliferation by blocking important cell surface proteins such as the interleukin-2 receptor (CD25),32,33 the E-rosette receptor (CD2),34 or the T-cell receptor-associated antigen (CD3)35,36 may predominate.

A clue to the mechanism of action of ATG and ALG might be provided by the comparative laboratory analysis of a lot (ALG 97412) described as being relatively ineffective in clinical trials in aplastic anemia patients.17 Unfortunately, clinical data from Europe and the United States are contradictory in regard to the “activity” of this particular lot. In an American study (reviewed in 38), this lot led to a 31% rate of transfusion independence in 35 patients as opposed to a 69% response rate in a previous study of 29 patients by the same investigators using ALG EC162.4 However, use of lot 97412 in Basel resulted in response of 9 of 12 patients with severe or moderate aplastic anemia, similar to excellent results obtained with a previous active lot (ALG 15343) and a subsequent lot (ALG 181S). While the reason for the discrepancy between the American and European experience with lot 97412 will probably never be determined, it is difficult to conclude that this ALG lot was clinically ineffective.

Our studies were undertaken on the report of clinical inactivity of lot 97412.17 The results of our blocking experiments are similar to a published comparison of this “inactive” ALG lot and an active ALG lot (EC162)38; in particular, the relative amounts of antibodies specific for known T-cell antigens were similar in the two studies. While Smith et al38 concluded that the overall 2- to 5-fold decrease in antibody blocking activity of the “inactive” lot, rather than the absence of a single antibody specificity, might account for clinical inactivity, we found the “inactive” lot very similar to another known active ALG lot (15343). The immunostimulatory capacity of this “inactive” lot, as determined by blastogenesis assays and hematopoietin production by T cells was equivalent to or superior to active lots of ALG and ATG. Although the “inactive” lot was less cytotoxic at suboptimal concentrations and failed to plateau even at concentrations 4- to 8-fold greater than the concentrations required for most other lots, this pattern was similar to that observed with an active lot, 181S. There was greater lot to lot variability among ALGs than among ATGs (which were remarkably homogenous in their properties as tested), but even when viewed independently of the clinical data, our laboratory studies failed to show a unique property of lot 97412 not shared by other ALG lots.

A question that has not yet been addressed in clinical trials is the relative effectiveness of ATG versus ALG. Response rates to ATG have averaged about 50%,8 whereas the first American trial of ALG resulted in a response rate of 68%4 and more recent Swiss data have suggested response rates as high as 85% with ALG in combination with adjuvant therapies.5 If these response rates are truly different, they may reflect differences in patient selection, clinical management, the addition of high-dose corticosteroids and androgens, and the regimen for administration of the horse serum in addition to intrinsic differences in the preparations. Patients in Europe are often observed for several months for spontaneous recovery prior to therapy; the use of isolation and antibiotics is not uniform among centers; and androgens are employed with ALG more enthusiastically abroad2 than in the United States.19 In addition, there may be an advantage to administering horse serum in large doses over a short period of time (as in most ALG protocols), prior to the onset of serum sickness and more rapid clearance of horse IgG,19 rather than over 10 to 28 days (as in most ATG protocols). If intrinsic properties of ALG are responsible for higher response rates in clinical trials, our results would direct attention to proteins such as Leu 1 and HLA-DR, which are much better bound by ALGs than ATGs.

Monoclonal antibody therapy of aplastic anemia has not been successful.40,41 Another approach to more specific and less toxic immunotherapy of aplastic anemia would be the production of polyclonal antisera directed against a limited number of defined antigens present on target T cells. While the broad antibody specificities and multiple biologic properties of ATG and ALG have hindered the elucidation of their mechanism of action, comparison using these assays of ATGs and ALGs with more restricted antisera may ultimately be revealing.

ACKNOWLEDGMENT

We thank Dr Leonard Bielory for analyzing the protein concentration and purity of the various ATG and ALG lots, Dr N. Chariatte for providing details of the preparation of the different ALG lots, Mike Vail and James Wong for their assistance with the flow microfluorometry analysis, Dr Arthur Niemhuis for his helpful advice and critical reading of the manuscript, and Imogene Surrey for her help in preparation of this manuscript.
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