Fractionation of Antibodies to L-Cell Colony-Stimulating Factor by Affinity Chromatography

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Purified L-cell colony-stimulating factor (CSF) was coupled to cyanogen-bromide-activated Sepharose and used to selectively fractionate antibodies to this factor. With the use of a simplified two-step washing and elution technique, there was 50%–70% binding of the anti-CSF, with recovery of 60%–100% of the bound material. Both the native antiserum and purified anti-CSF fractions were inhibitory to murine granulocyte-macrophage colony formation. The purified antibodies contained only IgG and were reduced in protein concentration to 0.1% of the serum IgG values. These fractions should prove useful tools for the study of granulocyte and macrophage differentiation.

Several studies have shown that antisera to certain proteins can be specifically fractionated and purified by immunoabsorbent or affinity chromatography techniques. In these techniques, purified antigens are covalently bound to an insoluble matrix such as cyanogen bromide-activated Sepharose. Specific antibodies are adsorbed to the gel and subsequently eluted with high-molarity or low-pH conditions.

Recently, several groups have purified murine sources of colony-stimulating factor (CSF). In our laboratory similar techniques have been used for the purification of L-cell-derived CSF. The present studies were designed to couple this pure CSF to an immunoabsorbent gel and use the bound material for selective fractionation of antibodies directed against this factor. The findings reported here detail the techniques for binding CSF to Sepharose gel and for removing CSF antibodies from various rabbit antisera. Such purified antibodies may prove useful in a variety of studies of granulopoietic control mechanisms.

MATERIALS AND METHODS

Murine CSF was obtained by growth of L cells in serum-free CMRL 1066 tissue culture medium. The supernatant-conditioned medium, which contained approximately 2000 units (U) of CSF per milliliter, was concentrated 250-fold by ultrafiltration and subjected to ethanol precipitation. The supernatant from the 50% ethanol step was concentrated and applied to DEAE-cellulose. The CSF was eluted by a linear NaCl gradient and subsequently applied to concanavalin A (con-A) Sepharose. Two peaks of activity were obtained. Peak 1 was nonadherent to the con-A, whereas peak 2 was bound and eluted with a-methylglucoside. Both peaks of activity were further separated on Sephadex G-150. In the final purification step the CSF was separated from trace contaminants by ultracentrifugation using a 5%–15% sucrose gradient. The results from the purification of one 10,000-ml pool of CSF are shown in...
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Table 1. Summary of L-Cell CSF Purification

<table>
<thead>
<tr>
<th>Purification Step</th>
<th>Total Protein (mg)</th>
<th>Total Activity (CSF units × 10^14)</th>
<th>Specific Activity (CSF units/mg × 10^-4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conditioned medium</td>
<td>420</td>
<td>19.8</td>
<td>0.005</td>
</tr>
<tr>
<td>I. Ultrafiltration</td>
<td>57</td>
<td>16.8</td>
<td>0.29</td>
</tr>
<tr>
<td>II. Ethanol precipitation</td>
<td>48</td>
<td>19.7</td>
<td>0.41</td>
</tr>
<tr>
<td>III. DEAE-cellulose</td>
<td>14.1</td>
<td>17.3</td>
<td>1.23</td>
</tr>
<tr>
<td>IV. Con-A Sepharose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak 1</td>
<td>0.58</td>
<td>4.5</td>
<td>7.8</td>
</tr>
<tr>
<td>Peak 2</td>
<td>0.94</td>
<td>8.3</td>
<td>8.8</td>
</tr>
<tr>
<td>V. Sephadex G-150 (peak 2)</td>
<td>0.35</td>
<td>8.2</td>
<td>23.4</td>
</tr>
<tr>
<td>VI. Sucrose density gradient (peak 2)</td>
<td>0.15</td>
<td>7.9</td>
<td>52.6</td>
</tr>
</tbody>
</table>

Values are derived from a single 10,000-ml pool of L-cell CSF. Final recovery of the step-VI (peak-2) CSF was 49% of the starting activity.

In the initial study, 120 μg of highly purified step-V CSF (specific activity 1.2 × 10^5 U/mg protein) was coupled to 1 g of cyanogen-bromide-activated Sepharose 4B (Pharmacia). The CSF was equilibrated in 0.1-M NaHCO_3-0.5-M NaCl buffer, pH 8, containing 0.3% polyethylene glycol 4000 (PEG). All subsequent buffers in these studies, contained PEG; as previously reported, this prevented loss of CSF in dilute solutions by adsorption to glass or Millipore membranes. The Sepharose gel was activated with 200 ml of HCl (1 mM) for 15 min in a sintered glass funnel. The CSF (120 μg) was added to 1 g of the activated gel and constantly turned on a rotary mixer for 2 hr at room temperature. After overnight incubation at 4°C, the material was again mixed for several hours at room temperature. The resultant gel was centrifuged and washed twice with 5 ml of the bicarbonate buffer. The residual reactive groups on the gel were blocked by the addition of 1-M ethanolamine, pH 8, for 1 hr at room temperature. After centrifugation this step was repeated. Following the second wash with ethanolamine, the gel was poured into a column (0.8 × 5.0 cm) and washed with three sequential 5-ml volumes of 0.1-M sodium acetate-1-M NaCl, pH 4. The column was then washed with 0.1-M TRIS-1-M NaCl, pH 8, containing 0.3% PEG to remove all traces of unreacted protein. All solutions and buffer washes from the preceding coupling were concentrated and dialyzed against 0.1-M TRIS buffer for assay of CSF to determine the extent of CSF binding to the gel.

Antisera directed against L-cell CSF were dialyzed against 0.1-M TRIS-0.1-M NaCl buffer, pH 8, prior to application to the gel. In the initial experiments various amounts of dialyzed antisera were passed through the Sepharose column and rinsed with sufficient starting buffer to remove nonadsorbed proteins. This was monitored by sequential testing of the absorbance values on a Zeiss spectrophotometer at 280 nm. The degree of antiserum binding to the column was evaluated by assay of dilutions of the starting antibody and each eluted fraction against a standard source of CSF.

After the binding characteristics of the column were established, several different agents were individually tested for their ability to elute the bound antibodies by dissociation of the antigen-antibody complex. These included sodium chloride, sodium thiocyanate, sodium perchlorate, and guanidine hydrochloride. Typically, each was tested in concentrations ranging from 0.5 to 2.0 M; pH was varied from 4 to 6.

After the conditions of binding and elution of the antibodies were established with the smaller column, 473 μg of pure step-VI CSF (specific activity 5 × 10^5 U/mg protein) were bound to a larger column. This material, which derived from 12 liters of starting L-cell-conditioned medium, was coupled to 1.5 g of Sepharose gel and poured into a 1- × 5-cm column. As before, all coupling and washing buffers were assayed for CSF activity. Several different buffers were employed to elutiate the column. Initially it was believed that moderately high molarity would reduce nonspecific binding of proteins other than the CSF antibody to the column. However, TRIS-HCl buffers containing 0.5-M or 1-M NaCl markedly reduced antibody binding. After further study is was found that 0.1-M TRIS-HCl-0.1-M NaCl buffer,
pH 7.5, permitted optimal binding of the anti-CSF without contamination of the eluted antibodies by other proteins.

The studies with the small column employed 5–10-ml aliquots of antisera with neutralization titers of 1:64 to 1:256. In order to reduce the time required for passing large volumes of antiserum through the column, higher-titer antiserum was used with the larger column. Following dialysis against starting buffer, 3 ml of antiserum with a neutralizing titer of 1:2048 were applied to the gel and rinsed with a similar volume of buffer. Contaminating proteins were removed by continuous passage of 45–50 ml of starting buffer. Anti-CSF was eluted from the gel either by sequential use of 10-ml fractions of 0.5-M, 1.0-M, 1.5-M, or 2.0-M guanidine HCl in starting buffer adjusted to pH 4 or by single passage of 10 ml of the 2-M guanidine buffer. To minimize possible denaturation of the anti-CSF by the guanidine, eluates were collected with continuous stirring into 10 ml of starting buffer that was kept at 4°C. All binding, washing, and other elution studies were carried out at room temperature. Prior to assay for CSF, the resultant 20-ml eluates were concentrated by ultrafiltration to the volume of starting antiserum and repeatedly dialyzed against starting buffer using a PM 10 membrane (Amicon).

Both starting antiserum and guanidine eluates were tested by Ouchterlony gel diffusion using goat antirabbit serum and goat antiserum directed against rabbit IgG. Protein concentrations of the eluted antibody fractions were measured by a dye binding technique using Coomassie blue G-250.11

CSF activity was determined by the agar gel bioassay.12 Each culture plate contained 10⁵ CFU₅ mouse bone marrow cells immobilized in 1 ml of McCoy’s −0.3% agar. Three to five culture plates were prepared for each point to be assayed. After 7 days of incubation in a fully humidified 7.5% CO₂ atmosphere, colonies larger than 50 cells were scored with the aid of a dissecting microscope. Units of CSF activity were defined as the number of colonies stimulated by the L-cell CSF using the linear portion of the dose–response curve, which generally fell between 20 and 100 colonies per culture.

Colony morphology was examined in cultures stimulated by standard L-cell-conditioned medium and by two pure step-VI CSF preparations (peaks 1 and 2). Individual colonies were aspirated from the agar with a Pasteur pipette and stained with 0.4% orcein in 60% acetic acid. Colonies were examined on days 3, 4, and 7 of culture. Colony type was scored as granulocyte (more than 80% ring and segmented forms), mixed, or macrophage (more than 80% macrophages).

Antibody titers were determined by addition of 0.1 ml of doubling dilutions of antiserum or diafiltered antibody fractions to the L-cell standard. The titer of antibody was defined as that dilution, which by interpolation, caused a 50% decrease in colony formation from the standard L-cell CSF. Anti-CSF units were calculated by multiplying the reciprocal of the 50% antibody neutralization titer by the number of colonies stimulated with the standard CSF. Thus, by definition, anti-CSF units exceeded CSF units by a factor of two at equivalence. Owing to inherent variations in the bioassay, 50% neutralization titers for a single antiserum varied from one study to another. Accordingly, the antibody activities of all samples from a single column run were assayed in the same experiment.

Several studies were done to assure that inhibition of colony formation was due to anti-CSF rather than trace contamination by guanidine. In the initial studies 2-M guanidine buffer was dialyzed against 0.1-M TRIS-0.1-M NaCl-0.1-M glycine buffer and subsequently tested for inhibition. A second approach employed precipitation of the anti-CSF by sheep antirabbit IgG serum. Aliquots of purified antibody fractions were mixed with 1 ml of 5% normal rabbit serum (NRS) in TRIS buffer. The IgG was precipitated with 0.3 ml of sheep antirabbit IgG; controls were diluted with NRS and buffer. After centrifugation, supernatants were tested for residual inhibitory activity.

Both native antiserum and purified anti-CSF were tested for cross-reactivity in erythropoietin-dependent CFU-e cultures as previously described.13 Cultures were prepared using 0.8% methocel, 30% fetal calf serum, 1% deionized BSA, 0.35 units of erythropoietin, and 10⁵ murine marrow cells in 1 ml of alpha medium. Erythropoietin was kindly supplied by the Division of Blood Diseases and Resources of the National Heart, Lung, and Blood Institute, National Institutes of Health. After 42–48 hr of incubation in a 5% CO₂, 37% atmosphere, colonies of 8 or more cells were scored using an inverted microscope. The effect of anti-CSF was evaluated by adding 10% volumes of varying dilutions of antiserum or purified anti-CSF to each culture.

RESULTS

In the initial study, 120 µg of highly purified step-V CSF were coupled to 1 g of cyanogen-bromide-activated Sepharose. No CSF activity was detected in any of the coupling, blocking, or washing reagents after appropriate concentration and dialy-
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Table 2. Effects of Dissociating Agents on Anti-CSF Elution

<table>
<thead>
<tr>
<th>Dissociating Agent</th>
<th>pH 6</th>
<th>pH 5</th>
<th>pH 4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>6%</td>
<td>4.3%</td>
<td>9.1%</td>
<td>19.4%</td>
</tr>
<tr>
<td>NaSCN (2 M)</td>
<td>16.8%</td>
<td>9.2%</td>
<td>4.6%</td>
<td>30.6%</td>
</tr>
<tr>
<td>NaClO₃ (2 M)</td>
<td>9.1%</td>
<td>18.4%</td>
<td>7.2%</td>
<td>34.7%</td>
</tr>
</tbody>
</table>

Guanidine (pH 4.0) | 9.8% | 11.6% | 9.1% | 30.5% |

Values denote percentages of bound antibody activity eluted with reduction in pH or increase in molarity. Values were calculated from antibody neutralization titers using the agar-gel colony assay.

tration against TRIS buffer. Thus it appears likely that the entire 120 µg or 1.4 × 10⁶ units of CSF activity, were bound to the gel. In the initial antibody binding studies, two 5-ml aliquots of CSF antiserum (titer 1:128) were passed through the column and washed with 0.1-M TRIS buffer. CSF assays showed complete binding of both aliquots to the column, indicating that 1.7 × 10⁶ units of anti-CSF activity were adherent. Minimal antibody activity was recovered after washing the column with 0.5–1.0-M NaCl in 0.1-M glycine buffer, pH 2.7. In further studies, 5 ml of this antiserum were applied to the column and rinsed extensively with TRIS buffer. The degree of recovery of anti-CSF activity using various dissociating agents is shown in Table 2. These compounds showed differing degrees of antibody elution at pH 6, 5, and 4. The guanidine elution was accomplished by stepwise addition of increasing molarity using a constant pH 4. As shown, virtually all of the agents led to 20–35% recovery of the bound antibody fractions.

In a further study a pool of CSF antiserum with a titer in excess of 1:128 was applied to the gel and eluted with a single 10-ml passage of 2-M guanidine. Seventy-three percent antibody binding was observed, with 38% recovery of the bound antibody. To exclude the possibility that trace levels of guanidine may have influenced the results, 2-M guanidine was diafiltered against buffer and subsequently tested for CSF inhibition. Several samples were inhibitory to colony formation using 1:2 dilutions; however, further dilution abolished this effect. In two studies, purified anti-CSF was added to 5% normal rabbit serum and precipitated with sheep antirabbit IgG. Control samples showed colony inhibition in excess of

![Fig. 1. Sequential application of antiserum to a CSF-Sepharose column. In this study, 80%–84% of the antibody activity from each aliquot of antiserum was bound to the column. Of the total 13 x 10⁶ anti-CSF units applied from 100 ml of antiserum, 10.6 x 10⁶ units were adherent.](#)
1:16 dilution. After precipitation with the sheep antiserum, supernatants showed complete loss of antibody activity, with no inhibition against the L-cell standard.

Following these initial results, 473 µg of pure step-VI CSF were coupled to the activated Sepharose gel as described earlier. CSF assay of the various coupling and buffer solutions again showed no free CSF activity, indicating complete binding to the gel. To determine the extent of antibody binding to the gel, sequential aliquots of antiserum were added to the column, and the effluent was assayed for anti-CSF. As shown in Fig. 1, the first 50 ml of antiserum contained $6.5 \times 10^6$ anti-CSF units; 80% of this activity was bound to the column. Two further additions of 25-ml aliquots of antiserum showed 81% and 84% binding. Thus, of the total $13 \times 10^6$ units applied, $10.6 \times 10^6$ units were adherent. Twenty percent recovery of the bound antibodies was noted following a single 10-ml wash with 2-$M$ guanidine buffer.

In order to reduce the time for adherence of antibodies to the column, further studies were done with high-titer antiserum that had a 1:2048 neutralization titer in the bioassay. Because of low antibody recovery, the technique was modified to include 0.1-$M$ glycine in the 0.1-$M$ TRIS-0.1-$M$ NaCl, pH 7.5, buffer to stabilize the purified anti-CSF. Three milliliters of antiserum were dialyzed against starting buffer, applied to the column, and rinsed with 50 ml of buffer to remove nonspecific proteins. The column was eluted either with stepwise increments of guanidine dissolved in the starting buffer or with a single 2-$M$ guanidine wash. Each 10-ml eluate was collected into 10 ml of starting buffer and held at 4°C; dilution was accomplished by constant mixing on a magnetic stirrer. In five studies, antibody binding to the column ranged from 50% to 60%, with a total binding of 6–11 $\times 10^6$ units of anti-CSF activity. Total antibody recovery averaged 58% of bound activity (Fig. 2).

To determine if the variations in binding and recovery were due to antibody heterogeneity, two high-titer antisera were studied in detail. Each antiserum was divided into 10 aliquots of 3 ml each and processed as above. The nonadherent and

![Fig. 2. Binding and elution characteristics of high-titer antiserum. Values denote means ± SE from five experiments.](https://www.bloodjournal.org/content/9/5/1186/F1.large.jpg)
Table 3. Recovery of Purified Anti-CSF From Two High-Titer Antisera

<table>
<thead>
<tr>
<th>Anti-CSF Units</th>
<th>Rabbit 164 (titer 1:1740)</th>
<th>Rabbit 166 (titer 1:2860)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applied</td>
<td>$6.6 \times 10^6$</td>
<td>$1.1 \times 10^7$</td>
</tr>
<tr>
<td>Nonadherent</td>
<td>$1.7 \times 10^6$</td>
<td>$6.3 \times 10^6$</td>
</tr>
<tr>
<td>Recovered (2-M guanidine)</td>
<td>$4.7 \times 10^6$ (71%)</td>
<td>$5.2 \times 10^6$ (48%)</td>
</tr>
</tbody>
</table>

High-titer antisera were applied to the CSF column in 3-ml volumes, rinsed with 50 ml of buffer, and eluted with 10 ml of 2-M guanidine buffer. The nonadherent and adherent fractions from 10 column runs for each antiserum were pooled and assayed for anti-CSF activity. The values in parentheses indicate the percentages of starting antibody activity that were recovered in the guanidine eluates. Each purified fraction showed only a single precipitin line when reacted against either goat antirabbit serum or goat antirabbit IgG.

guanidine-eluted fractions from the 10 column runs were pooled, diafiltered, and assayed for anti-CSF. The results are shown in Table 3. Although the antiserum from rabbit 164 showed a lower anti-CSF titer, 84% of this antibody was bound to the gel, with 71% recovery of the starting antibody activity. Antiserum from rabbit 166 showed a higher neutralization titer; however, less of the antibody activity was bound and subsequently recovered in the guanidine eluate.

Further experiments were designed to assess the specificity of the anti-CSF. Dilutions of high-titer starting antiserum, nonadherent (low-affinity) antibody, and the guanidine eluate were tested for their effects on both erythroid and granulocyte-macrophage colonies. The starting antiserum had a neutralization titer in excess of 1:3200 against L-cell-derived colonies, but dilutions of 1:50 through 1:3200 had no effect on CFU-e growth. Similar results were noted with the nonadherent and guanidine-eluted anti-CSF preparations. Although markedly inhibitory to white cell colonies, no inhibition of erythroid growth was observed.

By Ouchterlony gel diffusion the guanidine eluates showed only a single precipitin line when reacted against goat antirabbit serum or goat antirabbit IgG serum (Fig. 3). Protein recoveries in two concentrated guanidine eluates were 0.6 and 0.8 µg/ml, which represented approximately 0.1% of the starting serum IgG concentration.

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**Fig. 3.** Ouchterlony gel diffusion analysis of anti-CSF serum and purified anti-CSF antibodies obtained by elution with 2-M guanidine. These were reacted against goat antirabbit serum (GARS) and goat antirabbit IgG (GARG).
Table 4. Colony Types Stimulated by L-Cell CSF

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gran.</td>
<td>Mix.</td>
<td>Mac.</td>
</tr>
<tr>
<td>Peak-1 CSF</td>
<td>92%</td>
<td>8%</td>
<td>0</td>
</tr>
<tr>
<td>Peak-2 CSF</td>
<td>100%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>L-cell-conditioned medium</td>
<td>not done</td>
<td>0</td>
<td>26%</td>
</tr>
</tbody>
</table>

Values denote percentages of granulocyte, mixed, or macrophage colonies after varying incubation times. The numbers of colonies ranged from 93 to 101 on day 4 and 109 to 126 on day 7 of incubation. Each time point is based on examination of 35–50 colonies.

In a pilot study the purified CSF antibody was coupled to a Sepharose gel and used to selectively remove the CSF from L-cell-conditioned medium. Purified antibody (0.46 × 10⁶ units of anti-CSF) was coupled to the gel and poured into a column 0.5 × 5 cm. In three studies, dialyzed serum-free L-cell CSF was applied to the column and eluted with 2-M guanidine. Using starting material that contained 1.2–1.8 × 10⁷ units of CSF activity, 50%–80% of the applied material was bound to the column. Guanidine recoveries of CSF ranged from 30% to 52% of the bound material. As yet, insufficient quantities of this CSF are available to determine the degree of purification.

To determine the types of colonies stimulated by L-cell CSF, agar cultures were examined after 3, 4, and 7 days of incubation. Ten plates each were stimulated by pure step-VI CSF (peak 1 or peak 2) and by L-cell-conditioned medium. The results are shown in Table 4. The colonies formed in response to the step-VI CSF preparations were granulocyte type on day 3, mixed type on day 4, and predominantly macrophage type on day 7. The same general pattern was observed with the L-cell standard, except that in this study colonies were exclusively of the granulocyte type on day 4.

DISCUSSION

These studies show that CSF can be readily coupled to an immunoabsorbent gel and used to fractionate rabbit antibodies to this factor. In the initial studies several dissociating compounds were able to elute between 20% and 30% bound material. After slightly modifying the technique, a column that contained 473 µg of pure CSF bound 6–11 × 10⁶ units of antibody activity. By use of a two-step procedure in which the column was sequentially rinsed with buffer and 2-M guanidine, 58% of the bound antibody activity was recovered. Further experiments with two high-titer antisera showed that as much as 84% of antibody activity was bound, with nearly complete recovery in the guanidine eluates.

Although it was not possible from these studies to determine the purity of the fractionated antibodies, the immunodiffusion studies indicated that the eluted materials contained only IgG. With a reduction in protein concentration to approximately 0.01% of the starting serum value or 0.1% of the IgG concentration in the serum, it seems reasonable to suggest that the eluted materials represent only the specific CSF antibody fraction.

These observations show that each antiserum contains a spectrum of antibodies with varying affinities for CSF. Repetitive loading of the immunoabsorbent column does not indicate all-or-none binding characteristics. Rather, a proportion of antibodies was bound with each sequential addition of antiserum (Fig. 1). More-
over, a moderate amount of the bound antibodies was eluted with the extensive buffer washes necessary to completely remove unbound serum proteins. The extent of this elution appeared to vary with different antisera (Table 3). These findings indicate desorption of the antibody fractions with low binding constants. Stepwise additions of increasing concentrations of guanidine yielded antibody activity in the 0.5-M, 1.0-M, 1.5-M, and in some studies 2-M fractions. Moreover, elution using various pH buffers yielded similar results (Table 1). These results suggest varying antibody affinities for the bound CSF. Furthermore, selective fractionation of antibodies can separate those with low, medium, and high binding characteristics.

Stanley has recently postulated that at least two types of murine CSF are responsible for in vitro colony formation. Based on the morphology of colonies on the seventh day of culture, the stimulating factors have been termed G (granulocyte) or M (macrophage) CSFs. Although it is well recognized that colonies stimulated by L-cell CSF are composed primarily of macrophages on the seventh day of culture, earlier studies showed that colonies are almost exclusively granulocytic 3–4 days after incubation. The present studies confirm this observation. Using either two pure preparations of CSF or the L-cell-conditioned medium, there was early granulocyte maturation, with later conversion to macrophage colonies. Thus it would seem that both purified CSF and anti-CSF may be useful materials for in vivo study of granulocyte and macrophage differentiation.

The use of the separation techniques described herein will be expected to produce substantial amounts of purified antibody for in vivo experiments. Although injection of CSF antiserum has caused nearly complete inhibition of diffusion-chamber granulopoiesis in mice, repetitive administration of CSF antibody to intact mice has not reduced granulocyte differentiation. In part, this may result from limitations imposed by the amount of antiserum that can be injected into mice. With the ability to prepare highly purified antibody fractions, it may be feasible to administer considerably greater quantities of anti-CSF such that endogenous CSF may be completely neutralized.

Earlier studies showed that it was possible to detect CSF-producing cells in mice using an indirect immunofluorescence technique. At that time, neither pure CSF nor purified antibody was available as a control for blocking and binding studies. With these suitably purified reagents it may now be possible to identify the cellular sources of CSF in vivo and investigate the nature of hemopoietic cells in the marrow that bind CSF.

Recently developed radioimmunoassays for CSF have proved highly useful in the assay of most, but not all, sources of murine CSF. From the present studies it is clear that a spectrum of antibodies with varying binding affinities exists in each antiserum. Perhaps by careful fractionation of these antisera with stepwise or linear gradients, it may be possible to enhance the antibody fractions that cross-react with subtypes of murine CSF. Such materials may prove useful in providing optimal conditions for immunoassay of various kinds of CSF activity.

These studies also show that the purified CSF antibodies can be linked to Sepharose and used to selectively bind CSF by “reverse affinity.” Such antibody-bound columns are usually less successful than antigen-bound columns owing to the presence of multiple antibody binding sites on antigens but only one or two antigen combining sites on each antibody molecule. Despite such considerations, these studies show that small quantities of CSF (approximately 10⁵ units) do bind to and
elute from a column containing $0.46 \times 10^6$ anti-CSF units. By coupling much larger amounts of purified antibody to such gels, it may be possible to purify various sources of CSF by this rapid affinity chromatographic technique.

REFERENCES

Fractionation of antibodies to L-cell colony-stimulating factor by affinity chromatography

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