Antibody Stimulation of Hemopoietic Progenitor Cells

By Richard M. Crapper and John W. Schrader

Antisera were raised by immunizing rabbits with cloned lines of murine hemopoietic progenitor cells (P cells) that depended on the presence of a specific hemopoietic growth factor, persisting cell-stimulating factor (PSF), for their growth and survival. The unabsorbed antiserum was inhibitory, but after absorption with murine spleen cells and the mastocytoma, P815, significant stimulation of both P cell growth and thymidine incorporation was evident. IgG antibodies isolated from the antiserum by staphylococcal protein A chromatography or further purified by diethylaminoethyl anion exchange chromatography, ammonium sulphate precipitation, and gel filtration using Sephacyr S-300 were responsible for the stimulation. The absorbed antiserum promoted the survival of normal murine bone marrow cells in liquid culture over a four-day period, and the inclusion of IgG antibodies in agar cultures of normal bone marrow promoted the in vitro survival, over a 48-hour period, of cells capable of subsequently generating, in the presence of a source of PSF, colonies of neutrophils, macrophages, and megakaryocytes. It is postulated that the antibodies act by stimulating the PSF receptor on both factor-dependent cell lines and normal myeloid progenitor cells.

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NATURALLY OCCURRING antibodies specific for cell surface receptors have been implicated in the pathogenesis of a number of diseases, the antibodies having either stimulatory effects as exemplified by antibodies against the TSH receptor in thyrotoxicosis,12 or inhibitory effects exemplified by antibodies against the acetylcholine receptor in myasthenia gravis13 or the insulin receptor in insulin-resistant diabetes mellitus.9 Moreover, antibodies deliberately raised against specific cell-surface receptors for hormones such as insulin, epidermal growth factor, or acetylcholine have been shown to either mimic or inhibit the action of the respective hormone.5 7 Given that a series of hormone-like factors regulate the growth and function of hemopoietic cells, the question arises as to whether antireceptor antibodies could play a role in the pathology of hemopoietic diseases. Here we report observations that are consistent with this notion and demonstrate the antibody-mediated stimulation of murine hemopoietic cells.

Lines of murine hemopoietic cells, termed persisting or P cells because of their characteristic persistent in vitro growth, can be established from normal bone marrow using a growth factor produced by T lymphocytes and termed P cell-stimulating factor (PSF).8 9 Analysis of the biological activity of the purified molecule indicates that PSF stimulates multiple lineages of hemopoietic progenitor cells including those of neutrophils, macrophages, T-dependent mast cells, and megakaryocytes, as well as pluripotential hemopoietic stem cells.6 The term PSF is synonymous with colony-forming unit–spleen (CFU-S)-stimulating activity,10 mast cell growth factor,11 hemopoietic cell growth factor,12 burst-promoting activity,13 histamine-producing cell-stimulating factor,14 and multi-colony–stimulating factor,15 all of which are produced by the same sources and have similar, if not identical, properties. Protein sequencing16 17 and gene cloning experiments18 19 indicate that PSF is closely related to interleukin-3 (IL-3), a lymphokine defined by the induction of the enzyme 20-α-hydroxysteroid dehydrogenase,16 PSF differing from IL-3 in having an additional six N-terminal amino acids.16 17

The populations of P cells that we and others11 20 23 originally described can be reproducibly grown from precursors present at characteristic frequencies in normal lymphoid and hemopoietic tissue and special sites such as the gut mucosa. These cells have been shown to be the in vitro counterparts of the atypical or T cell-dependent subset of mast cells present in lymphoid and mucosal tissue, and their growth in vitro probably reflects their normal physiological response to the T cell lymphokine PSF.24 In keeping with this notion, they are diploid and cease growth after a few months in culture. Less frequently, cell lines that are also PSF dependent arise but, apparently as the result of some genetic change, are immortal; these have the characteristics not only of mast cells25 but also of megakaryocytes,26 neutrophils,26 27 erythroid cells,28 or multipotential stem cells.26 Because these cell lines can be readily cloned and grow rapidly in the presence of PSF, they form an ideal source of material for the analysis of the interaction of PSF with its target. In an attempt to raise antibodies against the receptor for PSF, we immunized rabbits with a PSF-dependent murine hemopoietic cell line that has both mast cell and megakaryocytic features and is termed R6-X.8

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MATERIALS AND METHODS

Factor-Dependent Cell Lines

Lines of P cells were generated from normal mouse bone marrow by culturing $10^5$ cells per millilitre in the presence of 3% WEHI-3B-conditioned medium (W3CM), as a source of PSF. Three particular cloned lines, R6-X, which give rise to cells with the morphological appearance of both mast cells and megakaryocytes, was used for the immunizations. This factor-dependent cell line also forms the basis of a sensitive assay for PSF. In brief, 500 R6-X cells in 8 μL of medium are plated out in the wells of Terasaki trays and 2-μl aliquots of the sample to be tested are titrated out serially in duplicate twofold dilutions. The trays are incubated upside-down for three days, pulsed with $[^3H]$-thymidine, and incorporation of $[^3H]$-thymidine is determined.

Production and Absorption of Immune Serum

For the immunizations, approximately $10^6$ R6-X cells were emulsified in complete Freund’s adjuvant and injected into rabbits in multiple subcutaneous sites. Injections of fresh R6-X cells ($10^6$) in saline were repeated at monthly intervals; after three to five injections, the serum was tested for inhibitory and stimulatory activity on R6-X cells. Inhibitory activity was measured by adding a constant amount of W3CM to the R6-X cells and titrating out the rabbit serum. Absorption of serum was performed by mixing 0.5 mL of the serum with $2 \times 10^6$ BDF1 spleen cells and agitating the suspension for 30 minutes at 4°C. After centrifugation, the serum was collected and the absorption was performed a further two times, using fresh spleen cells. The same serum was then absorbed another three times in the same way using the mastocytoma P815. After final absorption and centrifugation, the serum was sterilized by filtration.

Purification of Rabbit Immunoglobulin

Aliquots of 0.5 mL of the immune rabbit serum were applied to a Sepharose column conjugated with protein A (Pharmacia, Piscataway, NJ), 10 mL, equilibrated with phosphate-buffered saline (PBS), pH 7.3. Nonbinding fractions were collected, and the column was eluted, using an acetate buffer (pH 4) in 0.18 mol/L NaCl; the protein A binding fractions were determined and collected by ultraviolet (UV) absorption at 280 nm. The binding and nonbinding fractions were concentrated to the starting volume and dialyzed against PBS.

To purify the IgG fraction further, 20 mL of immune rabbit serum was applied to the protein A column and the binding fractions were pooled, dialyzed against 0.01 mol/L Tris HCl, pH 8, and chromatographed on diethylaminoethyl (DEAE)-Sephacel anion exchange column (Pharmacia) equilibrated with 0.01 mol/L Tris HCl, pH 8. Elution was performed with a linear gradient of NaCl (0 to 1 mol/L), the protein being determined by UV absorption at 280 nm. The elution profile obtained was similar to that previously reported for protein A-purified rabbit IgG. The IgG-containing fractions were pooled and concentrated by precipitation with 50% NH₄SO₄. The precipitate was redissolved in 3 mL of PBS and applied to a Sephacryl S-300 column (Pharmacia) equilibrated with PBS. A single symmetrical peak was eluted with PBS and identified, using UV absorption at 280 nm.

Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

To assess the homogeneity of the immunoglobulin preparation, samples were analyzed by SDS-PAGE according to the method of Laemmli. Slab gels 150 x 110 x 0.8 mm of 10% acrylamide (3:1 acrylamide to bis acrylamide) were overlaid with a stacking gel of 4.8% acrylamide. Samples (2 to 5 μg) were boiled for five minutes in SDS sample buffer with or without dithiothreitol. Standards were a mixture of phosphorylase b, mol wt 95,000; bovine serum albumin, mol wt 68,000; ovalbumin, mol wt 43,000; carbonic anhydrase, mol wt 30,000; and soybean trypsin inhibitor, mol wt 20,000. Gels were stained with Coomassie blue and destained with 12% ethanol and 70% acetic acid in water.

Bone Marrow Colony Assays

Colonies are scored on day 7. Whole plate staining was performed on a 1-mL volume in agar (0.3 vol/vol of agar, PBS. A single symmetrical peak was eluted with PBS and identified, using UV absorption at 280 nm. The elution profile obtained was similar to that previously reported for protein A-purified rabbit IgG. The IgG-containing fractions were concentrated to the starting volume and dialyzed against PBS.

RESULTS

Stimulation of Factor-Dependent Cell Lines by Immune Rabbit Serum

Serum from rabbits immunized with these R6-X cells had a marked inhibitory effect on the growth of P cells (Fig 1A). However, after sequential absorptions with syngeneic spleen cells and the mastocytoma P815, the inhibitory activity was greatly decreased (Fig 1B), suggesting that it was caused by antibodies against surface structures common to these cell types. Because studies based on both the binding of the iodinated factor and quantitative absorption of PSF activity have shown that PSF receptors are absent on spleen cells and P815 cells, these data indicated that the inhibitory activity that was removed by absorption with these cell types was not caused by antibodies directed against the PSF receptor.

At high dilutions, unabsorbed serum stimulated a small amount of $[^3H]$-thymidine incorporation by P cells (Fig 1C). However, after removal of much of the inhibitory activity by absorption with spleen cells and P815 cells, higher levels of thymidine incorporation were observed at higher concentrations of serum (Fig 1C). To exclude any nonspecific stimulation from the absorption process, normal rabbit serum that had been absorbed in the same manner was titrated out as a control (Fig 1C). No stimulation was seen. Comparisons with a standard preparation of tenfold concentrated medium that had been conditioned by the myelomonocytic leukemia WEHI-3B (W3CM) and had been assigned a value of $6.4 \times 10^4$ U/mL of PSF, indicated that the stimulatory activity of the absorbed antiserum was equivalent to $8 \times 10^3$ U/mL of PSF (Fig 1D). In the case of the antiserum, the slope of the titration curve was much shallower than with the W3CM, suggesting that inhibitory factors were still present in the serum. The stimulatory activity was manifest not only on the line used for immunization...
Fig. 1. (A) Inhibitory effects of normal (o—o) and immune (e—e) rabbit serum on the stimulation of R6-X cells by a 1:50 dilution of tenfold concentrated WEHI-3B-conditioned medium (W3CM) (Δ, mean ± SEM). (B) Inhibitory effect of same serum sample as in A (e—e), and after absorption with splenocytes (Δ—Δ) (0.5 mL of serum absorbed three times with 2 × 10^9 BDF1 spleen cells), or after further absorption with P815 (O—O) (three further absorptions of the 0.5 mL of serum with 5 × 10^8 P815 cells). (C) Stimulatory effects of unabsorbed (o—o), splenocyte absorbed (Δ—Δ), and splenocyte plus P815 absorbed (O—O) immune rabbit serum on the [3H]-thymidine incorporation by R6-X cells. The maximal stimulation by titrated normal serum absorbed with spleen and P815 (Δ) and the maximally absorbed immune rabbit serum (O—O).

but also on homogeneous populations of P cells that correspond to T cell-dependent mast cells (data not shown). The absorbed serum did not stimulate thymidine incorporation in T cell growth factor-dependent T cell lines specific for azobenzenearsenonate (data not shown).

To investigate whether the stimulatory effect of the absorbed antiserum on [3H]-thymidine incorporation by P cells was also associated with an increase in their number, ten R6-X cells were added to each of 12 Terasaki wells containing duplicate titrated amounts of the absorbed antiserum. By day 4 in the wells containing 10% and 5% by volume of absorbed antiserum, there were 28 ± 9 and 27 ± 6 cells, respectively (mean ± SD). In wells containing absorbed normal rabbit serum or medium alone, there were no viable cells. Although significant proliferation of R6-X cells was not evident beyond a dilution of the absorbed immune antiserum of 1:1,280, maintenance of viability was still present up to a dilution of 1:20,480.

**Agglutination of R6-X by the Absorbed Immune Antiserum**

We also examined the specificity of the absorbed IgG in agglutination assays over a 12-hour period in Terasaki wells. There was no agglutination of spleen cells or thymocytes, P815 was agglutinated to a titer of 1:640, and the R6-X cells were agglutinated to a titer of 1:20,480.

To exclude involvement of agglutination in the stimulatory mechanism, R6-X cells were cultured in agar (0.3%) at low concentration (200 per milliliter) in the presence of 10% absorbed antiserum. Inspection after six hours revealed only single cells; by day 2, however, approximately 30 clusters of between two to six cells were evident in cultures supplemented with the absorbed antiserum. In contrast, in control cultures supplemented with absorbed normal serum or medium alone, there were no viable cells. Thus, the proliferation and survival induced by the antiserum was not dependent on cell agglutination.

**Stimulation by Immune Serum Is Caused by Immunoglobulin**

To establish that the stimulatory activity was caused by immunoglobulin and not by some other factor in serum, we passed the antiserum over a Sepharose column coupled with staphylococcal protein A, collected the protein A-binding and nonbinding fractions and concentrated these to the initial volume. These were then assayed for PSF activity (Fig 2). It is evident that the majority of the PSF activity in the antiserum was present in the protein A binding fraction. In rabbit serum, this fraction consists almost exclusively of the IgG class of antibodies.

Further purification of the IgG was performed by means of DEAE ion exchange chromatography, NH_4SO_4 precipitation at 50% saturation and S-300 gelfiltration. A single protein peak eluted from the DEAE
column at 0.12 to 0.17 mol/L NaCl. This protein was all precipitated, using 50% saturated NH₄SO₄, and was applied to a gel filtration column. There was one peak of protein that eluted at a volume corresponding to a mol wt of approximately 1.5 x 10⁶. SDS-PAGE, 10% under nonreduced conditions, of an aliquot from the eluate from the Sephacryl S-300 column, showed a single band corresponding to the immunoglobulin component, with no evidence of lower mol wt proteins (Fig 3).

To determine whether the stimulatory activity copurified with IgG through this purification process, aliquots of the eluates from the protein A, DEAE, and gel filtration columns were adjusted to a concentration of 2 mg/mL in PBS and 1 mL of each sample was absorbed serially three times with 5 x 10⁶ BALB/c spleen cells. These absorbed, purified preparations of immunoglobulin were assayed for PSF activity by tritium-labeled thymidine incorporation. As shown in Table 1, there were no significant differences between the specific activities of the three preparations.

**Antibodies Do Not Stimulate Production of PSF or Emergence of Autonomous Variants**

Experiments were performed to exclude the possibility that the action of the immune immunoglobulins involved the stimulation by some unknown mechanism of the emergence of factor-independent variants. Such variants have been observed to emerge at low frequencies (of the order of 10⁻⁵) from the R6-X line (S. Schrader, J.W. Schrader, manuscript in preparation). R6-X cells (4 x 10⁶) were cultured in 200-μL volumes for 1, 2, or 4 days in the presence of 0.1, 0.3 and 1.0 mg/mL of the protein A-binding fraction of the absorbed immune rabbit immunoglobulin. After the period of culture, the cells were washed three times in HEPES-buffered Eagle's medium (HEM) and then resuspended in 10 μL of fresh medium in Terasaki wells at 5 x 10⁶/mL, either alone or with titrated amounts of W3CM as a source of PSF. R6-X cells that had been passaged in W3CM were washed and set up in parallel. Under all these conditions, the R6-X cells remained PSF-dependent and died in the absence of PSF and responded as usual to W3CM. Figure 4 shows the results, demonstrating the normal PSF dependence of R6-X cells that had been incubated for four days in the presence of 1 mg/mL of the protein A-binding fraction of the absorbed immune serum.

Another possibility raised by the observation of the emergence from R6-X of rare autonomous variants that produced PSF was that the rabbit serum was in some way stimulating the production of PSF from the R6-X cells. In an attempt to exclude this possibility, the supernatants were collected from R6-X cells that had been stimulated by immune rabbit serum for 1, 2, or 3 days. These supernatants were passed over a protein A column to remove residual antibodies, and

<table>
<thead>
<tr>
<th>IgG Concentration (mg/mL)</th>
<th>Protein A Binding</th>
<th>DEAE Elution</th>
<th>Gel Filtration</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.40</td>
<td>1.546 ± 63</td>
<td>1.844 ± 79</td>
<td>1.768 ± 111</td>
</tr>
<tr>
<td>0.20</td>
<td>1.162 ± 52</td>
<td>1.143 ± 87</td>
<td>1.036 ± 102</td>
</tr>
<tr>
<td>0.10</td>
<td>678 ± 83</td>
<td>721 ± 55</td>
<td>645 ± 75</td>
</tr>
<tr>
<td>0.05</td>
<td>341 ± 34</td>
<td>461 ± 61</td>
<td>442 ± 65</td>
</tr>
<tr>
<td>-</td>
<td>98 ± 35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Immune rabbit serum was purified by sequential steps, the IgG containing fractions at each step being identified by UV absorption at 280 nm. A 1-μL aliquot of each of the IgG fractions, at 2 mg/mL, was absorbed with BALB/c spleen cells and stimulatory activity on R6-X cells was determined at the indicated final concentrations. Results of one typical experiment are presented; data points represent means ± SEM of [³H]-thymidine incorporation.
the nonbinding fractions were assayed for PSF after concentration to one tenth of the volume of the original supernatant. No activity was detected.

**Immune Rabbit Serum Stimulates Normal Bone Marrow**

We next investigated whether the absorbed antiserum stimulated fresh bone marrow cells. The addition of 0.1 mL of absorbed immune antiserum to 1-mL cultures of $10^6$ bone marrow cells resulted in an increase in the number of viable cells present after four days. A mean of $3.6 \times 10^5$ cells per well was present in wells to which the absorbed antiserum had been added, compared with $9.8 \times 10^5$ cells in wells containing medium alone or $9.3 \times 10^4$ cells in wells containing absorbed normal rabbit serum. Cytocentrifuged preparations of the cells from the cultures stimulated with absorbed antiserum revealed numerous mitotic figures and many large immature cells, some of which showed features of differentiation along the myeloid or erythroid lineages into promyelocytes or erythroblasts (Fig 5). Similar conclusions were reached from experiments in which normal bone marrow cells were plated out into the wells of Terasaki trays containing titrations of the absorbed antiserum or normal rabbit serum, control cultures contained W3CM or medium alone. Figure 6 shows the result of a representative assay scored by counting viable cells at day 6. It can be seen that there was a significant increase in viability in cultures supplemented with the antiserum or W3CM.

The presence of mitotic figures and clusters of blast cells within cultures of bone marrow supplemented with the absorbed antiserum suggested that hemopoietic progenitor cells were being stimulated by the antibody. However, the addition of absorbed antiserum or the protein A binding fraction (up to 0.1 mL) to 1-mL agar cultures of bone marrow cells (up to $10^6$ cells per milliliter) did not result in colony formation. This result was not surprising, as the colony assay is known to be at least tenfold less sensitive than the

**Fig 4.** R6-X cells that had been in continuous passage in W3CM (●—●) were compared with cells that had been cultured for four days in the presence of PAB immune rabbit immunoglobulin (○—○). Well-washed R6-X cells from the two sources were added to the wells of Terasaki trays containing titrated amounts of W3CM. The means (± SEM) for the cells from W3CM (●) or from the immune serum (○) are also shown.

**Fig 5.** May-Grünwald-Giemsa-stained cytocentrifuge preparation of C57BL/6 bone marrow cells that had been cultured for four days at $10^6$ cells per milliliter in the presence of 10% absorbed immune rabbit serum. Note the large blast cells and one cell in mitosis.

**Fig 6.** Titration of absorbed antiserum on C57BL/6 bone marrow cells. Bone marrow cells, $10^6$ cells in 10 µL of medium were added to the wells of Terasaki plates containing dilutions of the absorbed immune serum. The cultures were incubated for four days, and numbers of viable cells were scored visually after the addition of 5 µL of 0.5% eosin (●). Also shown are the results for absorbed and titrated normal rabbit serum (○) and W3CM (●) and the mean ± SEM for medium alone (□).
standard PSF assay in detecting PSF. Therefore, as a
more sensitive test of whether the absorbed antiserum
affected colony-forming cells, we investigated whether
the antibody could maintain the survival of hemopoietic progenitor cells in vitro so that they could respond to a subsequent exposure to optimal concentra-
tions of PSF. Normal bone marrow cells were
plated out in agar in the presence of absorbed immune antiserum, protein A-binding, or nonbinding fractions, or normal serum, protein A-binding fraction, W3CM, or medium alone, and incubated for 48 hours. To
detect surviving colony-forming cells, W3CM (10%) was then added as a source of PSF, and colonies were
scored after a further five days in culture. The results
indicated that the presence of the absorbed immune antiserum in the first 48 hours stimulated a signifi-
cantly greater survival of cells capable of subsequently
generating colonies. Similar results were found with
the protein A binding but not the non–protein A-
binding fraction of the absorbed antiserum (Table 2).
Staining of these colonies revealed that they contained
mainly granulocytes and monocytes, although there
were some colonies of megakaryocytes. These experi-
ments thus showed that the addition to cultures of IgG antibodies from the absorbed antiserum promoted the survival of hemopoietic progenitor cells capable of subsequently forming colonies.

Table 2. Stimulation of Colony-Forming Cells in Normal Bone
Marrow by IgG Antibodies in Immune Rabbit Serum

<table>
<thead>
<tr>
<th>Growth Factor Added</th>
<th>Day 0</th>
<th>Day 2</th>
<th>No. of Colonies</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.5 ± 0.4</td>
</tr>
<tr>
<td>W3CM 0.2%</td>
<td></td>
<td>W3CM 10%</td>
<td>2.6 ± 0.9</td>
</tr>
<tr>
<td>W3CM 1%</td>
<td></td>
<td>W3CM 10%</td>
<td>28.6 ± 5.6</td>
</tr>
<tr>
<td>Absorbed antiserum, PAB 1 mg/mL</td>
<td>W3CM 10%</td>
<td>68.4 ± 4.4</td>
<td></td>
</tr>
<tr>
<td>Absorbed antiserum, PAB 0.5 mg/mL</td>
<td>W3CM 10%</td>
<td>15.5 ± 3.5*</td>
<td></td>
</tr>
<tr>
<td>Absorbed antiserum, PAB 0.25 mg/mL</td>
<td>W3CM 10%</td>
<td>15.6 ± 1.1†</td>
<td></td>
</tr>
<tr>
<td>Absorbed antiserum, PAB 0.1 mg/mL</td>
<td>W3CM 10%</td>
<td>8.4 ± 1.8*</td>
<td></td>
</tr>
<tr>
<td>Absorbed antiserum, NB 0.5 mg/mL</td>
<td>W3CM 10%</td>
<td>3.0 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>Absorbed antiserum, NB 0.1 mg/mL</td>
<td>W3CM 10%</td>
<td>1.8 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Absorbed normal serum, PAB 0.5 mg/mL</td>
<td>W3CM 10%</td>
<td>2.3 ± 0.8</td>
<td></td>
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</table>

DISCUSSION

These data show that serum from rabbits immu-
низированных R6-X фактор-зависимых муринских клеток стимулирует
both the proliferation and survival of cloned factor-dependent cell lines. Moreover, the addition of antibodies from absorbed immune sera to populations of normal bone marrow cells resulted in blastogenesis of immature cells and enhanced maintenance of viable cell numbers and enhanced survival of colony-forming cells. The evidence that the stimulatory activity demon-
strated here involves IgG antibodies is strong. Thus, the activity remained associated with this antibody
fraction through four sequential purification steps,
each involving separation by a different physicochemical parameter. Furthermore, at least in the case of the cloned cell lines, the action of the antibody must have been directly upon the cell that was stimulated. It remains possible, although unlikely, that the effects seen on normal bone marrow are mediated by an intermediate cell or cells.

A number of considerations suggest that the stimu-
ulatory effect of these antibodies involves binding of
antibody to the PSF receptor. There was no evidence for alternative mechanisms such as the possibility that the immune serum acts by stimulating the emergence of factor-independent cell lines or the production of
PSF by cells that could subsequently respond to it. Another piece of evidence against the notion raised by the emergence of autonomous variants from R6-X, were data that the absorbed immune antiserum also stimulated other P cell lines that do not give rise to
factor-independent variants. The absorbed immune antiserum agglutinated R6-X to a high titer (1:20,480), a 32-fold higher concentration being required to agglutinate the mastocytoma P815, whereas spleen and thymus cells were not agglutinated at all. It is important that the stimulatory effects of the absorbed immune antiserum on the survival of R6-X cells were also evident at titers up to 32-fold higher than those required to agglutinate receptor-negative P815 cells. Thus, the structure recognized by the stimulatory antibodies appeared to be present on R6-X cells but not on P815 cells (or spleen cells or thymocytes); thus, its distribution correlates with that of the PSF receptor established in experiments based on both the binding of the iodinated factor and quantitative absorption.

Certainly, the PSF receptor is the obvious candidate for a structure that mediates specific stimulation and is
shared by the two broad categories of cells stimulated by the antibodies, namely P cells and normal hemo-
poietic progenitor cells. Formal proof that antireceptor antibodies are involved will require purification and amino acid sequencing of the receptor and the demon-
stration that antibodies against corresponding synthetic peptides have stimulatory or inhibitory activities.

It has been reported that an ATP regenerating system enhanced the survival of PSF dependent lines of hemopoietic progenitor cells, raising the possibility that in the present experiments, the purified antibodies could be circumventing the normal stimulation of these cells by PSF. However, unlike the results reported with the ATP regenerating system, in the present experiments the antibodies did not merely support the survival of P cells, but also stimulated thymidine incorporation and cell growth and, furthermore, stimulated normal hemopoietic cells.

The present antisera should prove useful in isolation and characterization of the PSF receptor or—less likely—of some other structure involved in the stimulation of hemopoietic cells. The demonstration that antibodies can stimulate hemopoietic progenitor cells raises the possibility that a number of hematologic disorders may involve the inhibitory or stimulatory effect of antibodies on hemopoietic progenitor cells.

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