Splenic Granulocytopoiesis and Production of Colony-Stimulating Activity in Lymphoma and Leukemia

By Peter L. Greenberg and Susan M. Steed

Spleen cell production of granulocyte-macrophage colony stimulating activity (CSA) and colony forming capacity (CFU-GM) from 59 patients with Hodgkin’s and non-Hodgkin’s lymphoma, acute (AML) and chronic myeloid leukemia (CML), and control subjects was quantified to evaluate local cellular potential for modulating splenic granulocytopoiesis. Mononuclear spleen cell conditioned media stimulated myeloid CFU-GM by human nonadherent marrow target cells. In contrast to conditioned media produced by marrow and peripheral blood cells, the vast majority of spleen CSA was generated by nonadherent lymphoid cells rather than adherent monocycte cells. The nonadherent cells producing CSA were non-T cells (assessed by sheep erythrocyte rosetting), with 98%±2% CSA produced by the nonrosetted fraction (B lymphocytes and null cells), and had a peak density heavier than that of granulocytic precursor cell proliferation.

During embryonic life the human spleen is a site of active hemopoiesis, whereas in adults, blood formation is usually restricted to the bone marrow. The spleen is a site of extramedullary hemopoeisis in adults during severe hemopoeitic stress and in patients with a variety of neoplasms including leukemia, myeloproliferative disorders, and lymphomas. The mechanism whereby such extramedullary hemopoeisis occurs is not known.

Studies in experimental animals and humans have demonstrated that spleen cells provide microenvironmental influences and humoral substances capable of stimulating hemopoiesis in vivo and in vitro. Lymphoid and monocycte cells from peripheral blood and marrow provide colony stimulating activity (CSA-GM), the humoral substance necessary for granulocyte-monocycte progenitor cells (CFU-GM) to undergo proliferation and differentiation in vitro. Alterations in levels of CSA production and marrow CFU-GM occur following various perturbations, including stimulation with antigens and lymphocyte mitogens. Substances inhibitory for in vitro human granulocytopoiesis are also produced by these cells.

In order to evaluate factors involved in providing local cellular potential for modulating splenic granulocytopoiesis in man, we characterized the cells in spleen producing CSA and quantified levels of splenic CSA and CFU-GM in patients with malignant lymphomas and leukemia. Specific cytochemical staining of histologic sections was performed to assess the degree of granulocytopoiesis in these spleens. Urinary CSA output was determined following splenectomy to evaluate the contribution of the spleen to this source of CSA.

MATERIALS AND METHODS

Spleen Cell Production of CSA

Immediately after splenectomy, a portion of the spleen (an approximately 8 20 g slice) was placed in modified McCoy’s medium containing 15% fetal calf serum (FCS) and finely minced, producing a single cell suspension. Supernatant cells were obtained after Hypaque-ficoll gradient centrifugation as previously described. Cell viability, assessed by trypan blue dye exclusion, was generally >93%. Spleen conditioned medium containing CSA was obtained by plating the buoyant mononuclear cells (supernatant cells from the Hypaque-ficoll gradients, density ~ 1.077 g/cm³) at concentrations varying from 1 100 x 10⁶ cells/ml McCoy’s medium with FCS and 0.5 mM 2-mercaptoethanol in tissue culture dishes (Falcon Plastics). These mediums were harvested after incubation for 7 days at 37°C in a humidified air—7.5% CO₂ incubator and stored at °20°C until use. Utilizing previously described methods conditioned mediums were tested for CSA using nonadherent buoyant normal human marrow cells as target CFU-GM in 1 ml single layer agar culture. The CSA present in 0.15 ml test

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conditioned mediums was compared to a stable human mononuclear leukocyte standard CSA source, prepared as previously described.\textsuperscript{21} Dilution curves of CSA (colonies formed) were analyzed by curve fitting computer programs to assess effective CSA concentrations. In three separate experiments 50% ± 7% (mean ± SE) of the spleen cells and 57% ± 8% of the CSA were recovered by this method. This, processing of the spleen cells did not lead to selective loss or gain of CSA-producing cells. A unit of CSA is that amount necessary for stimulating the formation of one colony in vitro.

Spleen cell CSA values were analyzed with respect to cellular CSA production and were also extrapolated for CSA production by the entire spleen (Table 1). The latter value was obtained for each spleen by the following formula

\[
\text{Spleen CSA, units} = \frac{\text{CSA U}}{20 \times 10^6 \text{spleen cells}}
\]

* No. cells per weighed spleen slice · spleen wt. (g)

As mentioned above, a consistent underestimate was made of splenic CSA values due to lack of complete recovery of all spleen cells after the processing procedures.

**Spleen CFU-GM**

We utilized the same methods for evaluating spleen CFU-GM as we previously described for assessing marrow CFU-GM.\textsuperscript{21} Human spleen cells (1 × 10\textsuperscript{6}), obtained as stated above, were plated in a 1 ml layer of 0.3% agar medium over a human leukocyte feeder layer. Colonies, counted after 7–10 days of incubation, consisted of groups of >50 cells and were granulocytic and monocytic cells at various stages of differentiation. Total splenic CFU-GM content was assessed by multiplying CFU-GM/10\textsuperscript{6} spleen cells by the number of cells per spleen as determined by the above formula.

**Adherence Separation**

Our adherence separation procedure has been previously described.\textsuperscript{21} A suspension of 10\textsuperscript{6} spleen cells was placed in 3 ml medium with 7.5% FCS in 60 × 10 mm plastic tissue culture dishes for 30 min at 37°C in a air-CO\textsubscript{2} incubator. The dishes were rinsed with medium, the nonadherent cells removed and the adherent and nonadherent cell fractions were incubated separately in 2 ml medium containing 0.5 mM 2-mercaptoethanol for 7 days to provide CSA. In 27 lymphoma patients 67% ± 7% (mean ± SE) of the buoyant mononuclear cells were nonadherent, determined by counting the difference between the total number of cells plated and the nonadherent cells recovered after washing the plates. Direct counting of the adherent cells in measured areas of the tissue culture dishes generally confirmed the calculated number of adherent cells in representative experiments. Thus, an average 33 × 10\textsuperscript{6} residual adherent cells (33%) was provided by the 10\textsuperscript{6} cells plated. As these cells were incubated in 2 ml of medium the concentration of adherent cells producing conditioned medium was 17 × 10\textsuperscript{6} cells/ml.

**Morphology and Cytochemistry**

The morphology of the adherent cells was determined by permitting the cells to adhere to glass cover slips in the tissue culture dishes. The cover slips were removed, washed with medium to remove loosely adherent cells, stained with Wright's-Giemsa and α-naphthyl acetate esterase (ANA esterase) stains and examined microscopically. The method of performing the ANA esterase stain for the identification of monocytes (diffuse cytoplasmic staining) has previously been described.\textsuperscript{21} The morphology of the nonadherent cells was assessed by staining cytotoxic preps preparations.

Histologic sections (5 μ thick) of the spleens were evaluated for granulocytopenia using hematoxylin-cosin, chloroacetate esterase (CAE esterase) for identifying granulocytes, pinacyanol erythrosinate for mast cells (that are also CAE esterase positive), and periodic acid-Schiff (PAS) stain to assess splenic architecture.\textsuperscript{22} When immature granulocytic cells could be identified in at least three 10 × 40 power fields, granulocytopenia was considered to be present. Generally, foci of several single immature granulocytic cells were observed scattered in the central compartment of the red pulp and marginal zones surrounding the lymphoid follicles or the subcapsular regions. Granulocytic precursors were distinguished from mast cells by pinacyanol erythrosinate staining, as well as by the more intense cytoplasmic staining of the mast cells with PAS. Utilizing these cytochemical techniques the CAE esterase positive mononuclear cells were identified as immature granulocytic cells.\textsuperscript{23} “Whole plate” staining of agar cultures was performed to specifically determine the type of colonies generated by spleen conditioned medium. The agar gels in tissue culture dishes were immersed in water, removed by rinsing, floated onto glass slides and permitted to dry at room temperature overnight. They were then placed in the appropriate fixative (see references 22–25). The gels were stained cytochemically with CAE esterase, ANA esterase, or luxol fast blue (for identifying eosinophils).\textsuperscript{26}

**Separation of Lymphocyte Subpopulations**

To determine the proportion of sheep erythrocyte rosetted (E-rosetted) T lymphocytes, 5 × 10\textsuperscript{6} nonadherent buoyant spleen cells/ml (obtained by Hypaque-ficol separation as described above) in Hank's balanced salt solution 50% FCS (absorbed, heat-

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**Table 1. Spleen Cell Generation of Colony Stimulating Activity (CSA) and Myeloid Colony Forming Cells (CFU-GM)**

<table>
<thead>
<tr>
<th></th>
<th>CSA Units</th>
<th>CFU-GM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Spleen wt. (g)</td>
</tr>
<tr>
<td>Controls</td>
<td>3</td>
<td>210 ± 105*</td>
</tr>
<tr>
<td>Lymphoma (+)</td>
<td>13</td>
<td>186 ± 17</td>
</tr>
<tr>
<td>Lymphoma (−)</td>
<td>35</td>
<td>166 ± 10</td>
</tr>
<tr>
<td>AML remission</td>
<td>3</td>
<td>167 ± 40</td>
</tr>
<tr>
<td>CML</td>
<td>5</td>
<td>5,165 ± 333*</td>
</tr>
</tbody>
</table>

* p < 0.05
† p < 0.003 compared to control values. Other values are not significantly different from the controls.

‡ Mean ± SE.

TN = colonies too numerous to count.
inadequate) were mixed with an equal volume of a fresh 3% sheep erythrocyte solution (Colorado Serum Corp., Denver, Col.) as previously described.27 The cell mixture was layered on Hypaque-Ficoll then centrifuged at 23°C for 30 min at 2000 rpm. The light density (non-rosetted B lymphocytes and null cells) and pelleted (rosetted T lymphocytes) cells were separately recovered. The two cell fractions were examined by hemocytometer to evaluate their relative homogeneity, then separately incubated at a concentration of 5 x 10⁶ cells/ml for 7 days to prepare CSA in conditioned medium as described above. After the mass rosetting procedure, the recovery of nonadherent buoyant cells in 9 separate experiments was 92% ± 6%. The cells were ANA esterase negative and were >95% viable (trypan blue dye testing). The relative purity of cells obtained by E-rosetting was independently shown by T cell cytotoxicity and surface immunoglobulin (B lymphocyte) staining (performed by Dr. S. Strober, Stanford University Medical Center).28 The nonadherent buoyant cell fractions from two patients had 60% and 66% E-rosette positive cells and cytotoxicity testing showed 66% and 77%, respectively, to be T lymphocytes. The rosetted cell fractions in these patients had only 3% and 1%, respectively, cells bearing surface immunoglobulin.

**Albumin Density Separation Procedure**

Our procedure for performing continuous gradient equilibrium centrifugation utilizing bovine serum albumin for evaluating density distribution patterns of marrow cells has previously been described.29,30 Linear albumin gradients were generated in the density range 1.055–1.082 g/cc cm. Gradients containing 1–8 x 10⁶ mononuclear spleen cells were centrifuged for 40 min at 5000 g at 4°C. Cell fractions were collected at 4°C by upward displacement and centrifuged after dilution with McCoy’s medium. The cell pellets were resuspended in medium and plated to make adherent and nonadherent conditioned medium for CSA provision as described above. Refractive index (Bausch and Lomb Abbe-3L Refractometer) was utilized for evaluating fraction density.

**Urinary CSA Output**

Our method for evaluating the daily urinary output of CSA has previously been described.30 We utilized the calcium phosphate adsorption and concentration technique, which permits quantitation of urinary CSA essentially free of inhibitory substances. Dilution curves were obtained with processed urine samples stimulating C57BI mouse marrow CFU-GM. Normal values for daily urinary CSA output are 68 ± 21 x 10³ (mean ± SD) U.30

**Patients**

A portion of the spleen from 48 previously untreated individuals with Hodgkin’s and non-Hodgkin’s lymphomas undergoing splenectomy was obtained. Following laparotomy, pathologic staging indicated that 20 patients were stage I, 12 were stage II, 12 were stage III, and 4 were stage IV. The spleens showed histologic involvement with lymphoma in 13 cases (27%) and were negative in 35. Forty-four patients had Hodgkin’s lymphoma, the remaining 4 patients (3 with negative spleens) had non-Hodgkin’s lymphoma. Histologic classifications of the Hodgkin’s lymphoma patients were 76% nodular sclerosis, 12% mixed cellularity, and 12% undifferentiated. Uninvolved portions of the spleen were utilized for our studies from patients with tumor-positive spleens. Spleens from 3 patients with acute myeloid leukemia (AML) in remission and 5 patients in the chronic phase of chronic myeloid leukemia (CML) were studied.

Control spleens were obtained from three patients undergoing splenectomy for traumatic rupture, splenic vein thrombosis, and incidental to abdominal surgery. The patients had essentially normal peripheral blood counts except for six individuals with thrombocytosis. For comparison of histologic sections depicting granulopoiesis in the spleens obtained for our in vitro studies, cytochemical stains of sections were also examined from six consecutive patients with normal spleens removed following traumatic rupture.

Statistical analyses were performed utilizing the chi-square test with Yates correction, Student’s t test, and the Wilcoxon two sample two-tailed rank test. Values were considered significantly different if p < 0.05.

**RESULTS**

**Spleen CSA**

Dose response curves indicated that spleen CSA production was directly related to cell concentration, with plateau values occurring at concentrations of 20–100 x 10⁶ buoyant mononuclear cells/ml (Fig. 1). Significantly increased amounts of CSA were produced by cells from lymphoma and AML remission spleens in comparison with normal spleens (Table I). No significant difference in spleen weights or CSA per spleen was noted in these patient groups. No significant difference in CSA production per cell or per spleen was noted when comparing spleens involved (positive) or uninvolved (negative) with tumor, except in stage III patients where positive spleens had increased CSA production per cell (p < 0.05). Moderate but insignificant increments in cellular and splenic CSA were noted in patients with more advanced stages of their diseases (e.g., 249 ± 87 versus 191 ± 25 U and 368 ± 119 versus 281 ± 56 U, stage IV versus stage I, respectively). No differences in CSA were found between Hodgkin’s and non-Hodgkin’s lymphoma patients, histologic subtypes among the

Fig. 1. CSA production by buoyant mononuclear spleen cells. Dose response curves show mean ± SE values from control subjects, lymphoma patients with (+) and without (−) splenic involvement by tumor, AML in remission, and CML.
lymphoma patients, or between lymphoma and AML remission patients. In contrast to these patients, CSA production was undetectable from conditioned medium prepared from unseparated spleen cells of the five patients with CML (see below).

**Subpopulations of Spleen Cells**

The buoyant spleen cells were separated into adherent and nonadherent fractions. The vast majority of spleen CSA (69%–87%) was generated by the nonadherent cells from 3 normal, 17 lymphoma with negative spleens, 6 lymphoma with positive spleens, and 2 AML remission patients (Fig. 2). Nonadherent spleen cell production of CSA was significantly higher than that generated by adherent cells from these patients (p < 0.001, 0.001, and 0.05, respectively). Further shown in Fig. 2, unseparated mononuclear spleen cells had decreased CSA production in comparison with equal numbers of cells in the nonadherent fraction in lymphoma and control patients. The decrease was significant for the lymphoma patients with tumor-negative spleens (p < 0.01). Prostaglandin E, which is produced by adherent cells, is capable of inhibiting granulocytic colony formation. However, addition of indomethacin (an inhibitor of prostaglandin synthesis) to the incubation mixture did not increase CSA production (Table 2). Figure 3 shows concentration-related increments in CSA production by adherent, nonadherent and total cells from lymphoma patients with tumor-negative spleens.

As shown in Figs. 1 and 2, no CSA was detected in conditioned medium prepared from unseparated or nonadherent cells from CML patients, whereas CSA was made by the adherent cells alone (Fig. 2). This suggests that a soluble inhibitor(s) was elaborated by the nonadherent cell population. To determine whether the inhibitor was directed against the CSA-producing cells or against normal CFU-GM target cells, mixing experiments were performed in which medium conditioned by nonadherent CML cells was added to cultures containing nonadherent buoyant normal marrow target cells stimulated by CSA from leukocyte conditioned medium. Conditioned mediums from cells of two CML patients added to the normal CSA source diminished normal colony formation by 24% and 67% (Table 3), indicating the presence of an inhibitor against normal CFU-GM. Dialysis against water for 3 days did not remove the inhibitory activity.

The percentage of nonadherent mononuclear spleen cells which were E-rosette positive (T lymphocytes) were 41% ± 12% (n = 2), 49% ± 4% (n = 5), and 67% ± 2% (n = 3) for control, Hodgkin's lymphoma (4 tumor-negative and 1 tumor positive spleen) and AML remission patients, respectively. The proportion of T lymphocytes in spleens of AML remission patients was significantly increased (p < 0.01) in comparison with that of patients with Hodgkin's lymphoma or the Hodgkin's lymphoma patients and control subjects combined. Utilizing cells obtained by mass rosetting 98% ± 2% of the CSA was produced by the nonrosetted cell population (Fig. 4), demonstrating that cells other than T lymphocytes constitutively contributed essentially all of the splenic CSA. ANA esterase stains were negative in both of these cell populations indicating lack of contamination of these cell fractions by monocytes.

**Table 2. Effect of Indomethacin on Mononuclear Spleen Cell Production of CSA**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Mononuclear* Cells</th>
<th>Non-adherent* Cells</th>
<th>Adherent† Cells</th>
<th>Indomethacin‡ Mononuclear* Cells</th>
<th>Adherent† Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 1</td>
<td>353</td>
<td>397</td>
<td>0</td>
<td>220</td>
<td>0</td>
</tr>
<tr>
<td>No. 2</td>
<td>161</td>
<td>230</td>
<td>68</td>
<td>166</td>
<td>54</td>
</tr>
</tbody>
</table>

*20 × 10⁶ cells/ml.
†12 × 10⁶ cells/ml.
‡10⁻⁷ M.
MR Imaging of Thymoma: Radiologic-Pathologic Correlation

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MR images in 17 patients with surgically proved thymomas (12 malignant and five benign) were reviewed and compared with pathologic specimens. In nine cases, MR images of excised specimens also were made and evaluated. On T2-weighted images, 11 of 12 malignant thymomas had an inhomogeneous signal intensity, half with and half without a lobulated internal architecture. None of the five benign thymomas had a lobulated internal architecture, and they all had a moderately or slightly inhomogeneous signal. Cystic regions and/or hemorrhage were noted pathologically, and corresponded to areas of inhomogeneous high signal intensity seen on T2-weighted images. Examination of the excised specimens in malignant thymomas showed that the lobulated configuration seen in the tumors was caused by thick fibrous septa. Our experience suggests that, although calcification cannot be identified, MR is helpful in making a differential diagnosis of mediastinal tumors and in determining malignancy of thymoma.

Although several articles have described the MR appearance of thymic tumors [1, 2], detailed studies correlating the MR and pathologic appearances of these lesions have not yet been reported. Accordingly, we reviewed the MR images of 17 surgically proved thymomas and compared the findings with gross and microscopic findings noted in surgical specimens.

Materials and Methods

The study group comprised 12 patients with malignant thymoma and five patients with benign thymoma. The six women and 11 men were 36–75 years old (average, 55 years). All thymomas were excised, and a pathologic diagnosis was established.

Thymomas were classified as malignant when extracapsular invasion and/or pleural dissemination were seen at surgery, or when there was invasion of the capsule and/or cytologic atypia in microscopic specimens. The diagnosis of benign thymoma was made when features of malignancy were not found. Eight of 12 malignant thymomas showed macroscopic extracapsular invasion at surgery, and four had microscopic capsular invasion in the pathologic specimens. None showed pleural dissemination at surgery. Whereas two malignant thymomas showed obvious cytologic atypia, the other 10 showed no cytologic atypia.

Seventeen patients were examined with a 1.5-T superconductive MR unit (Signa, General Electric, Milwaukee, WI) or a 0.5-T superconductive unit (Resona, YMS, Tokyo, Japan). MR images were obtained with spin-echo T1-weighted imaging sequences, 500–1000/20–25 (TR/TE), and proton density-weighted and T2-weighted multiecho imaging sequences (1500–2500/20–25,50–100) by using ECG or peripheral pulse gating and respiratory compensation. The slice thickness ranged from 5 to 7 mm with a 2- to 5-mm interslice gap. MR and CT examinations were performed within 2 weeks before surgery. In nine thymomas (five malignant and four benign), T1-weighted (600/20) and T2-weighted (2000–3000/80–100) axial MR images of the excised specimens were obtained with the Signa unit.
in remission, and 3 with CML. Spleen weights in the lymphoma and AML patients were normal (164 ± 18 and 167 ± 40 g, respectively), whereas they were increased in the CML patients (1535 ± 438 g). Baseline daily urinary CSA output was decreased in lymphoma patients (29 ± 6 x 10^4 U, p < 0.001), normal in AML in remission (47 ± 34 x 10^4 U), and increased in the CML patients (336 ± 149 x 10^4 U). As shown in Fig. 6, marked increments in CSA output occurred in lymphoma and AML remission patients (537% and 742%, respectively) within 1–3 days post-

splenectomy that subsequently returned to basal values by 5–7 days. Patterns of urinary output did not differ in lymphoma patients with positive or negative spleens. A significantly lower increment in CSA levels (124%) was noted in the CML patients (p < 0.02) on day 2 following an initial decrease in CSA output on the first postoperative day.

**Splenic CFU-GM**

Splenic CFU-GM values are indicated in Table 1. No significant differences were noted in splenic CFU-GM concentration or content between lymphoma, AML patients, and control subjects. These values were both significantly decreased in stage IV lymphoma patient in comparison with stage I patients (0.9 ± 0.5 colonies/10^6 cells versus 5.4 ± 1.3 colonies/10^6 cells [p < 0.01] and 2.9 ± 2.2 x 10^4 colonies versus 13.7 ± 3.1 x 10^4 colonies per spleen [p < 0.02]). Markedly increased CFU-GM values were found in spleens from CML patients (p < 0.05). The CFU-GM content in the control spleens is comparable to that previously found in peripheral blood but only 0.1% of that present in bone marrow (Table 5). The incidence of CFU-GM in splenic vein blood, assessed in 7 patients with Hodgkin’s lymphoma, was 2.3 ± 1/10^6 nucleated cells plated. These values did not differ significantly from the CFU-GM incidence in spleens of these patients. No CSA was detected in splenic vein plasma from 7 individuals (4 lymphoma and 3 CML patients) when
tested against nonadherent buoyant human marrow CFU-GM.

**Histology of Spleens**

Histologic sections of the spleens were evaluated for morphological evidence of splenic granulocytopoiesis using the CAE esterase stain. Mature granulocytes were present within the red pulp of all spleens and were considered to be cells in transit. However, of interest was the finding of discrete foci of immature mononuclear granulocytic cells as detected by CAE esterase cytoplasmic positivity in 82% of the 45 lymphoma spleens examined. These cells were present in 25 of 32 lymphoma spleens without and 12 of 13 spleens with tumor, all 3 AML remission patients, 2 of 3 control spleens and in the 6 normal spleens examined for comparative purposes. Negative pinacyanol staining showed that the CAE esterase positive cells were not mast cells. The foci of several single granulocytepoietic cells were found in paranodular locations of the red pulp, predominantly in the marginal zones and splenic cords (Fig. 7) and also in the subcapsular region. A positive correlation was noted between extent of disease and the presence of splenic granulocytopoiesis. Twenty-eight of the 29 patients with stages II, III, and IV lymphoma had this morphological finding in comparison with only 14 of 21 patients with stage I disease (p < 0.02). No evidence of leukemic infiltration was found histologically in the spleens obtained from patients with AML in remission. All CML spleens had gross infiltration with a spectrum of differentiating granulocytic cells (see Table 4 for the morphology of CML spleen cell suspensions).

**DISCUSSION**

Our study has described the contribution of the spleen for human granulocytopoiesis and production of myeloid regulatory substances. We have characterized the cells responsible for myeloid CSA and have quantified alterations of in vitro parameters of granulocytopoiesis in spleens of patients with lymphoma and leukemia. We have demonstrated that the bulk of CSA produced by human spleen cells is generated by nonadherent, relatively dense, ANA esterase negative, E-rosette negative cells. This indicates that the predominant spleen cells producing CSA are null cells and/or B lymphocytes. Concentration-dependent increments in CSA production also occurred, albeit of decreased values, with adherent cells (Fig. 3). The observed phenomena that lower CSA levels were produced by total unseparated spleen cell relative to nonadherent or T-depleted spleen cells appear to be cell concentration dependent (i.e., explained by the lower concentration of the more active CSA-generating cell population). No direct evidence was provided to implicate production of inhibitory substances by the adherent or T cells from normal, lymphoma, or AML remission spleens. Both B and T lymphocytes have previously been shown to produce CSA, with T lymphocyte CSA production being predominantly demonstrated after mitogenic stimulation. Our data regarding CSA production by nonadherent cells are similar to those from mouse spleen and differ from those from most other sites of the body. In peripheral blood and marrow the predominant CSA-producing cells are adherent cells, mainly monocyte-macrophages.

The finding of spleen cells from patients with untreated Hodgkin’s and non-Hodgkin’s lymphoma and AML in remission and higher CSA production than those from control spleens suggests that these systemic diseases or (as in the case of AML patients) prior therapy or complications of the disease may cause increased CSA production by spleen cells. Lymphocytes stimulated by various antigens associated with immunologic reactions produce CSA. Despite local spleen cell CSA production, the lack of CSA in spleen vein plasma could relate to the presence of the previously described lipoprotein inhibitors of granulocytopoiesis in human serum. The absence of detectable CSA from spleen conditioned medium of the CML patients appears related to a nondialyzable substance(s) produced by nonadherent spleen cells that markedly inhibited normal CFU-GM response to CSA (Table 3, Fig. 1 and 2). The high levels of CFU-GM and granulocytopoiesis morphologically in the CML spleens suggest diminished effect of the inhibitor on leukemic as opposed to normal myeloid cell proliferation. This leukemia-associated inhibitory activity has previously been demonstrated with marrow and spleen cells of CML patients. Prior reports have shown the presence of a nondialyzable inhibitor in serum of CML patients following splenic irradiation. However, methodology for these latter studies renders difficult direct comparison with our data. The relatively low proportion of lymphoid cells in CML spleens (Table 4) suggests that low production of stimulatory molecules could also contribute to our findings.

The finding of a normal proportion of E-rosette positive T lymphocytes in spleens of our patients with Hodgkin’s lymphoma relative to our control subjects is in good agreement with recent investigations by others. Of interest was the increased proportion of splenic T lymphocytes in our AML remission patients comparable to elevations previously shown in CML.
Fig. 7. (A) Immature mononuclear granulocytic cells (arrows) in the marginal zone surrounding a lymphoid nodule of the spleen are demonstrated by cytoplasmic staining with chloroacetate esterase. Magnification × 400. (B) Red pulp of the spleen showing chloroacetate esterase positive immature mononuclear granulocytic cells (arrows) and polymorphonuclear neutrophils (p) in the cordal regions. Nonstained lymphoid cells are also present. Magnification × 400.
Despite the presence of foci of immature granulocytic cells in spleen and splenic CFU-GM, the low content of splenic CFU-GM (0.1% in comparison with marrow, Table 5) and the absence of postplenectomy neutropenia indicate that in the control, lymphoma, and AML remission patients the spleen does not contribute appreciably to normal systemic granulocytopoiesis. The lack of more extensive splenic granulocytopoiesis (several single immature granulocytes per focus) despite active local CSA production may be due to the absence of appropriate local stromal influences necessary for stem cell preservation and proliferation. Prior studies in adult mice have demonstrated low CFU-GM frequency and diminished positive microenvironmental stimuli for granulocytopoiesis in spleen relative to marrow. In addition, increased monocytic relative to granulocytic differentiation of colonies in agar was noted with CSA from spleen conditioned medium from our patients in comparison with blood leukocyte conditioned medium. The similarity of peripheral and splenic vein blood CFU-GM incidence with that present within the spleens of our control and lymphoma subjects suggests that the splenic CFU-GM in these patients may be part of a circulating CFU-GM transit population. It should be noted that these individuals were hematologically normal and had received no recent chemotherapy. Studies are needed to determine whether the more extensive splenic granulocytopoiesis found under hemopoietic stress is associated with alteration of locally produced stimulatory factors. Markedly increased splenic CFU-GM content and granulocytopoiesis has been demonstrated in mice following chemotherapy-induced neutropenia. The decreased CFU-GM frequency in spleens of stage IV lymphoma patients parallels like findings in marrows of patients with advanced lymphomas. In CML, our observation of increased levels of splenic CFU-GM has previously been described, with data suggesting splenic contribution to leukemic granulocytopoiesis.

Our findings of the high incidence of foci of immature granulocytic cells in the marginal zones and red pulp of spleens of our patients with lymphoma, AML in remission and control subjects is likely due to use of specific cytochemical staining for identification of mononuclear granulocytes (CAE esterase). Accurate identification of these cells in routinely prepared sections stained with hematoxylin-eosin is difficult and their relatively low numbers in spleen has led to their being frequently overlooked. Other workers have previously demonstrated the value of this approach for analyzing structural and cellular elements of the spleen. The marginal zone is a junctional area between the white and red pulp wherein many branching arterioles empty and blood is filtered. It is a major site for differential distribution of antigen, macrophages, and lymphocytes. Cords of reticulum-containing tissue separating the sinuses in the red pulp then receive the arteriolar supply and trap a variety of blood cells and their precursors. B and T lymphocytes are intermixed in the marginal zone. Thus, our finding of the localization of early granulocytic cells in this splenic region could relate to blood flow patterns and/or proximity to CSA-producing cells (null cells or B lymphocytes). Microenvironmental influences causing compartmentalization of hemopoietic foci within murine spleens have previously been demonstrated, including the nonuniform and nonrandom distribution of CFU-GM.

Postplenectomy increases in urinary CSA output occurred acutely in patients with lymphoma and AML in remission. Subsequent return of CSA output to basal levels was noted within a week, indicating that the spleen is not a major source of urinary CSA in these patients. These postoperative CSA alterations in urinary CSA levels have previously been shown in patients following other surgical procedures. Urinary CSA levels are elevated in CML as the disease progresses, and were also increased preoperatively in the patients in this study. In the CML patients, an initial decrease without a significant postoperative rise in urinary CSA was demonstrated, suggesting that the spleen in CML contributes to production of urinary CSA. The mechanism underlying this difference in splenic contribution to urinary CSA in these differing patients groups is not known. Although urinary CSA has low stimulatory potential for human marrow colony formation in vitro relative to mouse marrow, it can cause appreciable stimulation of human leukemic cell proliferation. The possible pathophysiologic relevance of the splenic production of this source of CSA remains to be clarified.

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Splenic granulocytopoiesis and production of colony-stimulating activity in lymphoma and leukemia

PL Greenberg and SM Steed