Absence of c-kit Receptor and Absent Proliferative Response to Stem Cell Factor in Childhood Burkitt's Lymphoma Cells

By Jörg Tomeczkowski, Andreas Beilken, Detlef Frick, Britta Wieland, Andrea König, Martin H. Falk, Alfred Reiter, Karl Welte, and Karl-Walter Sykora

The cytokine stem cell factor (SCF) synergizes with interleukin-7 (IL-7) to enhance the proliferation of pre-B cells. To examine the role of SCF and its receptor, c-kit, in the pathogenesis of pediatric Burkitt's lymphomas (BL), we investigated the expression of SCF and c-kit in BL cells and the mitogenic activity of SCF on BL cells. A panel of 13 BL cell lines and 7 fresh biopsy tumors was investigated. BL cells were stimulated either by Epstein-Barr virus (EBV) infection or by different reagents and cytokines, and expression of SCF and c-kit was studied on the mRNA level by Northern blot analysis and reverse-transcriptase polymerase chain reaction (RT-PCR), followed by Southern blotting. c-kit expression was also studied by fluorescence-activated cell sorting and by crosslinking of digoxigenin-labeled recombinant human SCF to the cell surface. Proliferation of BL cell lines was measured by \(^{3}H\)-thymidine incorporation. Low-level expression of c-kit mRNA was detected in 2 of 13 unstimulated BL cell lines and in 1 fresh BL tumor. One cell line showed upregulation of c-kit mRNA with A23187 and downregulation with phorbol myristate acetate. Neither c-kit nor SCF could be detected in any other cell line under any condition of stimulation as analyzed by Northern blot analysis, RT-PCR followed by Southern blot analysis, crosslinking, and immunofluorescence. No response to SCF was seen in \(^{3}H\)-thymidine incorporation assays. We conclude that most BL cells express neither SCF nor c-kit and that the low-level expression of c-kit in some BL cells most likely has no biological significance.

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THE PEDIATRIC non-Hodgkin's lymphomas (NHL) are a heterogenous group of malignancies of B- or T-cell origin that constitute 7% to 10% of all pediatric malignancies. In Europe and in North America, approximately 20% to 25% of childhood lymphomas are lymphoblastic lymphomas, half are small noncleaved cell lymphomas (mostly Burkitt's lymphoma [BL]), and the rest are predominantly large-cell lymphomas. With current chemotherapy protocols, between 65% and 90% of all patients with BL can achieve long-term disease-free survival.

The growth conditions of B-cell lymphomas and their dependence on growth factors are of interest, because cytokines are being added to chemotherapy protocols in patients with these conditions. The cytokines interleukin-2 (IL-2), interferon-β (IFN-β), granulocyte-macrophage colony-stimulating factor (GM-CSF), and granulocyte colony-stimulating factor (G-CSF) have been administered to patients with malignant lymphomas in an effort to modulate the therapeutic response or to alleviate the cytopenias of chemotherapy. The direct in vivo effects on BL cells of the therapeutically used cytokines have not been studied extensively. BL cells could be shown to express IL-2 receptors and could be activated to produce IFN-γ. G-CSF receptor were absent on BL cells, which could not be induced to express G-CSF receptors by different reagents. G-CSF did not influence proliferation. Lymphomas of different histologies show varying degrees of expression of IL-1, IL-2, IL-4, IL-5, IL-6, GM-CSF, and their receptors. The significance of this finding in the context of a complex cytokine network is often difficult to determine.

The recently described hematopoietic cytokine stem cell factor (SCF), also called kit ligand (KL), mast cell growth factor (MGF), or stem cell factor (SF), has entered clinical testing and has replaced by stroma cells and IL-7; the stroma cells can be used as an adjunct in the therapy of malignant diseases. SCF appears to be a potent mobilizer of peripheral blood progenitor cells (PBSCs), especially in conjunction with G-CSF. Nothing is known so far about a direct stimulatory or inhibitory effect of SCF on BL cells.

In combination with IL-7, SCF was found to be a potant stimulus at early stages of lymphoid development. Proiferation of murine bone marrow B-lineage cells is supported by stroma cells and IL-7; the stroma cells can be replaced by SCF for the proliferative effect but not for the further differentiation to Ig-expressing cells. SCF in combination with IL-6, IL-11, or G-CSF is able to maintain the B-lymphoid differentiation potential of primary bone marrow cultures. The combination of SCF and IL-7 provides a proliferative stimulus to murine B cells only after they have role in normal and leukemic hematopoiesis. Little is known about the growth factor requirements of BL cells in particular, although normal B cells of different stages of differentiation are known to respond to or require for their growth and differentiation the cytokines IL-1, IL-2, IL-4, IL-6, IL-7, or IL-12. Because of its stimulatory effects on early stem cells, SCF may become a candidate to support not only granulopoiesis but also thrombopoiesis in the setting of high-dose chemotherapy. In addition, CSFs are widely used in bone marrow transplantation and SCF appears to be a potent mobilizer of peripheral blood progenitor cells (PBSCs), especially in conjunction with G-CSF.

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Table 1. Origin, EBV Status, Karyotype, and Ig Expression of BL Cell Lines

<table>
<thead>
<tr>
<th>Cell Lines</th>
<th>Sex/Age (yr)</th>
<th>Ethnicity</th>
<th>EBV Status</th>
<th>Karyotypic Alterations</th>
<th>Ig Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHH-BL-1</td>
<td>F/14</td>
<td>White</td>
<td>Positive</td>
<td>ND</td>
<td>(\mu\lambda)</td>
</tr>
<tr>
<td>MHH-BL-2</td>
<td>M/5</td>
<td>Turkish</td>
<td>Negative</td>
<td>ND</td>
<td>(\mu\lambda)</td>
</tr>
<tr>
<td>IAPC-BL-2</td>
<td>M/7</td>
<td>White</td>
<td>Negative</td>
<td>t(8;22)</td>
<td>(\mu\lambda)</td>
</tr>
<tr>
<td>IARC-BL-2B95-8</td>
<td>M/7</td>
<td>White</td>
<td>Negative</td>
<td>t(8;22)</td>
<td>(\mu\lambda)</td>
</tr>
<tr>
<td>IARC-BL-2P3HR-1</td>
<td>M/7</td>
<td>White</td>
<td>Negative</td>
<td>t(8;22)</td>
<td>(\mu\lambda)</td>
</tr>
<tr>
<td>IARC-BL-16</td>
<td>F/5</td>
<td>Reunion</td>
<td>Positive</td>
<td>t(8;14)</td>
<td>(\mu\lambda)</td>
</tr>
<tr>
<td>IARC-BL-18</td>
<td>M/3</td>
<td>North-African</td>
<td>Positive</td>
<td>t(8;14)</td>
<td>(\mu\lambda)</td>
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<tr>
<td>IARC-BL-29</td>
<td>F/3</td>
<td>Reunion</td>
<td>Positive</td>
<td>t(8;14)</td>
<td>(\mu\lambda)</td>
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<tr>
<td>IARC-BL-31</td>
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<td>White</td>
<td>Negative</td>
<td>t(8;14)</td>
<td>(\mu\lambda)</td>
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<tr>
<td>IARC-BL-41</td>
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<td>White</td>
<td>Negative</td>
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<td>(\mu\lambda)</td>
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<tr>
<td>IARC-BL-4109-8</td>
<td>M/8</td>
<td>White</td>
<td>Negative</td>
<td>t(8;14)</td>
<td>(\mu\lambda)</td>
</tr>
<tr>
<td>IARC-BL-41P3HR-1</td>
<td>M/8</td>
<td>White</td>
<td>Negative</td>
<td>t(8;14)</td>
<td>(\mu\lambda)</td>
</tr>
<tr>
<td>IARC-BL-49</td>
<td>M/3</td>
<td>White</td>
<td>Negative</td>
<td>t(8;22)</td>
<td>(\mu\lambda)</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not done.

Table 2. Origin of Fresh BL Cells

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex/Age (yr)</th>
<th>Source of Cells</th>
<th>L3 Cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F/14</td>
<td>Ascites</td>
<td>45</td>
</tr>
<tr>
<td>2</td>
<td>M/10</td>
<td>Ascites</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>F/11</td>
<td>Pleural effusion</td>
<td>87</td>
</tr>
<tr>
<td>4</td>
<td>M/7</td>
<td>Pleural effusion</td>
<td>24</td>
</tr>
<tr>
<td>5</td>
<td>M/10</td>
<td>Ascites</td>
<td>21</td>
</tr>
<tr>
<td>6</td>
<td>M/7</td>
<td>Pleural effusion</td>
<td>21</td>
</tr>
<tr>
<td>7</td>
<td>M/9</td>
<td>Ascites</td>
<td>61</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not done.
Amersham, Braunshweig, Germany). Finally, the cells were harvested on glass fiber strips with an automated cell harvester and the incorporated radioactivity was determined by scintillation counting. All assays were performed in triplicate.

**Stimulation of BL cell lines.** To assess induced expression of c-kit or SCF mRNA in BL cell lines, the appropriate cytokine or reagent was added to the cells (1 × 10^6 cells/mL) in 75-cm² tissue culture flasks (Falcon 3024; Becton Dickinson, Meylan Cedex, France) containing 100 mL of BL culture medium with 10% FCS. The cytokines used were recombinant human IL-3 (Genzyme, Cambridge, MA) at a concentration of 34 pg/mL and recombinant kit or SCF mRNA in BL cell lines, the appropriate cytokine or reagent was added to the cells (1 × 10^6 cells/mL) in 75-cm² tissue culture flasks (Falcon 3024; Becton Dickinson, Meylan Cedex, France) containing 100 mL of BL culture medium with 10% FCS. The cytokines used were recombinant human IL-3 (Genzyme, Cambridge, MA) at a concentration of 34 pg/mL and recombinant kit or SCF mRNA in BL cell lines, the appropriate cytokine or reagent was added to the cells (1 × 10^6 cells/mL) in 75-cm² tissue culture flasks (Falcon 3024; Becton Dickinson, Meylan Cedex, France) containing 100 mL of BL culture medium with 10% FCS. The cytokines used were recombinant human IL-3 (Genzyme, Cambridge, MA) at a concentration of 34 pg/mL and recombinant human tumor necrosis factor-α (TNF-α; Knoll, Ludwigshafen, Germany) at a concentration of 5 ng/mL. BL cell lines were also treated with 2.5 μg/mL anti-Mg antibodies (Sigma, St Louis, MO), with 20 ng/mL phorbol 12-myristate 13-acetate (PMA; Sigma), or with 100 ng/mL calcium ionophore A23187 (Sigma). The Staphylococcus Cowan A cells (SAC; Pansorbin; Calbiochem, La Jolla, CA) were washed twice with PBS and added to the BL cells at a concentration of 0.1 vol%.

**Northern blot analysis.** Total cellular RNA from BL cell lines and fresh BL cells was extracted using the single-step acid guanidinium thiocyanate-phenol-chloroform extraction described by Chomczynski and Sacchi. MN. Fifteen micrograms of RNA per lane was passed through a nylon membrane (Hybond N; Amersham) using 3 mol/L NaCl, 0.3 mol/L sodium citrate, pH 6.5. 5X Denhardt's solution, 75 μg/mL denatured herring sperm DNA (Serva, Heidelberg, Germany), and 0.18% sodium dodecyl sulfate (SDS) at 56°C. The membranes were washed in 2× SSC containing 0.1% SDS at 58°C for 30 minutes, 0.1× SSC containing 0.1% SDS at 65°C for 30 minutes, and to a final stringency with 0.1× SSC containing 0.1% SDS at 73°C for 15 minutes. Autoradiography was performed with Hyperfilm MP (Amersham) at ~80°C using intensifying screens. The gels were stained with ethidium bromide to ensure that equal amounts of RNA were loaded.

Preparation of radiolabeled antisense RNA probes. The SCF cDNA probe was a 519-bp Sma I-Sph I DNA fragment cloned into the pGEM3-Vector, kindly provided by Dr K.M. Zebo (Amgen, Thousand Oaks, CA). The vector was digested with Sac I, and ^32P-CTP (Amersham) labeled antisense RNA was synthesized using SP6-RNA-polymerase (Boehringer Mannheim).

The c-kit probe was a 1.3-kb Sac I fragment derived from phc-kit-171 (ATCC clone, catalogue no. 59492; deposited by Axel Ullrich). It was subcloned into the Sac I site of pBluescript KS+ phagemid (pBS KS+; Stratagene). T3-RNA-polymerase (Promega, Heidelberg, Germany) was used to generate ^32P-CTP–labeled antisense RNA transcripts from the Ava II-digested vector.

**Polymerase chain reaction (PCR) after reverse transcription.** Single-stranded cDNA was synthesized from total cellular RNA in 40 μL reaction buffer containing 1 μg random hexamers and 20 U of Moloney murine leukemia virus reverse transcriptase (RT; BRL, Eggenstein, Germany). Inactivation of RT reaction was performed at 95°C for 5 minutes. A total of 2.5 μL of this reaction was diluted into 25 μL of PCR buffer containing 7.5 pmol of the upstream and downstream primers. The final MgCl₂ concentration was 1.5 mmol/L.

The set of SCF primers was designed to include exon 6 and therefore to discriminate between the mRNAs for soluble and membrane-bound forms of SCF. Control PCR reactions were performed with specific primers either for aldolase or 16S mitochondrial ribosomal RNA (16S RNA). The nucleotide sequences of the PCR prim-

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**Table 3. Primers and Conditions of PCR**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Location</th>
<th>Strand</th>
<th>Sequence (5'→3')</th>
<th>35 Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCF (Martin et al)</td>
<td>388-407</td>
<td>Sense</td>
<td>TGGAATAACGAGATGGTAG</td>
<td>55°C for 85 s, 72°C for 70 s, 94°C for 65 s</td>
</tr>
<tr>
<td></td>
<td>1125-1144</td>
<td>Antisense</td>
<td>TGGTACAGAAGAAGACCA</td>
<td>50°C for 85 s, 72°C for 70 s, 94°C for 65 s</td>
</tr>
<tr>
<td>c-KIT (Yarden et al)</td>
<td>653-673</td>
<td>Sense</td>
<td>TTGCTGGTGCTGGTTGTT</td>
<td>66°C for 85 s, 72°C for 70 s, 94°C for 65 s</td>
</tr>
<tr>
<td></td>
<td>1166-1186</td>
<td>Antisense</td>
<td>TGGCTTCTTGGTGCTTTTTA</td>
<td>66°C for 85 s, 72°C for 70 s, 94°C for 65 s</td>
</tr>
<tr>
<td>Aldolase (Tolan et al)</td>
<td>141-163</td>
<td>Sense</td>
<td>TTGCAAGGGCAGATCGGCTGCA</td>
<td>66°C for 85 s, 72°C for 70 s, 94°C for 65 s</td>
</tr>
<tr>
<td></td>
<td>561-583</td>
<td>Antisense</td>
<td>TAACCGGAGCAAGACATTGGGATT</td>
<td>94°C for 65 s</td>
</tr>
<tr>
<td>16S mt-rRNA (Eperon et al)</td>
<td>2347-2369</td>
<td>Sense</td>
<td>CAGTTAAAACCAGACTAACTGACA</td>
<td>50°C for 85 s, 72°C for 70 s, 94°C for 65 s</td>
</tr>
<tr>
<td></td>
<td>3077-3099</td>
<td>Antisense</td>
<td>GGGAGGAAATTGGAAGGATAG</td>
<td>94°C for 65 s</td>
</tr>
</tbody>
</table>
ers and the PCR conditions are shown in Table 3. PCR reactions were started by the addition of 1 U Taq polymerase (Perkin Elmer Cetus, Überlingen, Germany) in a DNA thermal cycler (Landgraf, Hannover, Germany).

**Southern blot analysis.** Products from the RT-PCR were separated by electrophoresis in a 1.8% agarose gel in TBE-buffer. DNA was denatured twice in a buffer containing 0.5 mol/L NaOH, 1.5 mol/L NaCl and subsequently neutralized twice in a buffer containing 0.5 mol/L Tris-HCl, 3 mol/L NaCl, pH 7.5. The gel was checked for the pH and blotted overnight onto a positively charged nylon membrane (Boehringer Mannheim) using 20× SSC as the transfer buffer. After transfer, the DNA was immobilized using an UV crosslinker (Stratalinker; Stratagene) as described in the Northern blot protocol. The membranes were washed briefly in H2O and air dried. Prehybridization and hybridization were performed using a nonradioactive labeling and detection system (Boehringer Mannheim). Digoxigenin (Dig)-labeled c-kit-specific internal oligonucleotide (upper panel). Mitochondrial 16S rRNA was amplified as positive control (lower panel). The length of size markers (M) is indicated in base pairs. Negative control reactions (−RNA and −cDNA) were performed by omitting RNA or cDNA template, respectively.

**Fig. 2.** RT-PCR of c-kit transcripts. Amplified 534-bp c-kit transcripts were separated on a 1.8% agarose gel and stained with ethidium bromide (middle panel). The gel was blotted and hybridized with a Dig-labeled c-kit−specific internal oligonucleotide (upper panel). Mitochondrial 16S rRNA was amplified as positive control (lower panel). The length of size markers (M) is indicated in base pairs. Negative control reactions (−RNA and −cDNA) were performed by omitting RNA or cDNA template, respectively.
c-kit receptor in Burkitt’s lymphoma

was found to have retained mitogenicity for M07e cells comparable to unlabeled rhSCF of the same concentration. M07e and BL cells (5 x 10^6 in 100 μL) were incubated with 15 μL (0.13 mg/mL) Digi-nhSCF for 1 hour on ice in the presence or absence of a 100-fold excess of unlabeled rhSCF. Cells were washed two times with ice-cold PBS to remove unbound SCF. Subsequently the cells were treated with 0.5 mmol/L of the bifunctional crosslinker disuccinimidylsuberate (DSS; Pierce, Geisenheim, Germany). The reaction was stopped after 45 minutes with 100 μL of 2 mol/L glycine, pH 6.8, and the cells were lysed with ice-cold PBS with 1% Triton X-100 (Serva). The soluble proteins were denatured for 15 minutes at 50°C in a buffer containing 0.06 mol/L Tris, 3% SDS wt/vol, 30% glycerol, 1 mmol/L phenylmethylsulfonyl fluoride (PMSF), and 15% β-mercaptoethanol and separated in a 7.5% SDS-polyacrylamide gel. The gel was blotted for 200 mA onto a Sartolon nylon membrane (Sartorius, Gottingen, Germany) using Bjerrum and Schäfer-Nielson’s buffer5 containing 0.06 mol/L Tris, 39 mmol/L glycine, 20% methanol, 0.0375% SDS, pH 9.5, as the transfer buffer. The membranes were washed twice in 20 mol/L Tris containing 0.1% Tween 20, 0.5 mol/L NaCl, pH 7.5, and incubated overnight in blocking buffer containing 100 mL Hank’s solution (8 g/L NaCl, 0.4 g/L KCl, 0.121 g/L Na₂HPO₄ x 2 H₂O, 0.1 g/L MgSO₄ x 7 H₂O, 1 g/L glucose x 1 H₂O, 0.1 g/L MgCl₂ x 6 H₂O, 0.35 g/L NaHCO₃, 0.3 g/L CaCl₂ x 6 H₂O, 0.01 g/L phenol red), 0.1% Tween 20, and 0.2 g/L BSA. The membranes were equilibrated two times with a buffer containing 20 mol/L Tris and 0.05% Tween 20, pH 7.5. Subsequently, the anti-Dig-AP antibody (Boehringer Mannheim) was diluted 1:10,000 in blocking buffer and the membranes were incubated for 1 hour; washed 7 to 10 times for 5 minutes in 20 mol/L Tris containing 0.1% Tween 20, 3% dry milk; and equilibrated two times with assay buffer (1% vol/vol diethanolamin, 1 mmol/L MgCl₂, pH 10.0). CSPD stock solution (11.6 mg/mL; Tropix) was diluted 1:100 in assay buffer. Membranes were incubated for 30 minutes with 1 mL of diluted CSPD solution at 37°C in a plastic bag. Excess buffer was removed completely and membranes were exposed to Cronex DDS 100 autoradiography films (Du Pont).

Flow cytometry. Cell samples were incubated for 20 minutes on ice with saturating amounts of monoclonal antibody (MoAb) in 1× PBS, 0.5% BSA, 0.1% sodium azide and were washed twice in 1× PBS, 0.1% BSA, 0.1% sodium azide. The MoAbs were stained by fluorescein isothiocyanate (FITC)-conjugated F(ab’). rabbit antihuman IgG (Dako, Hamburg, Germany) used as a second-step reagent. All samples were analyzed on a FACScan flow cytometer (Becton Dickinson, San Jose, CA). Primary MoAbs used were anti-CD10 (Calla; Becton Dickinson), anti-CD117 (c-kit; supernatant from clone 57A5D8B1 kindly provided by Dr H.-J. Bühring, Tübingen, Germany) and FITC-conjugated rabbit antihuman IgG, IgA, and IgM heavy and light chains (Behring, Marburg, Germany).

RESULTS

RT-PCR of either aldolase mRNA or 16S RNA. The quality of the generated cDNA was judged by amplification of the cDNA with aldolase specific primers that generate a 443-bp product or with 16S RNA specific primers that generate a 752-bp product. The amplification led to comparable amounts of DNA from all cell lines.

![Fig 3. RT-PCR of c-kit transcripts in A23187-stimulated BL cells. BL cells were stimulated with A23187 for 2, 5, 12, 24, and 48 hours. Amplified 534-bp c-kit transcripts were separated on a 1.8% agarose gel and stained with ethidium bromide (middle panel). The gel was blotted and hybridized with a labeled c-kit-specific internal oligonucleotide (upper panel). Mitochondrial 16S rRNA was amplified as positive control (lower panel). The length of size markers (M) is indicated in base pairs. Negative control reactions (–RNA and –cDNA) were performed by omitting RNA or cDNA template, respectively.](image-url)
RT-PCR and Southern blot analysis of SCF mRNA. SCF exists in both cell surface bound and soluble forms. SCF that is missing 28 amino acids in the extracellular domain is not protease cleavable and therefore remains bound to the cell surface. The 84 DNA basepairs that encode the 28 amino acids correspond precisely to the sixth exon of the human gene.8-14. The primer pair used in the present experiments discriminates between exon 6-positive and exon 6-negative transcripts. With exon 6-positive transcripts, it generates a 757-bp product. With exon 6-negative transcripts, it generates a 670-bp product. The amplification products were resolved in a 1.8% agarose gel and stained with ethidium bromide (middle panel). The gel was blotted and hybridized with a Dig-labeled internal oligonucleotide specific for c-kit (upper panel). Mitochondrial 16S rRNA was amplified as a positive control (lower panel). The length of size markers (M) is indicated in base pairs. Negative control reactions (-RNA and -cDNA) were performed by omitting RNA or cDNA template, respectively.

RT-PCR and Southern blot analysis of c-kit mRNA. Expression of c-kit receptor mRNA was investigated using RT-PCR followed by Southern blot analysis. The positive control cell line MO7e showed detectable amounts of c-kit mRNA by RT-PCR on an ethidium bromide-stained agarose gel, whereas MHH-BL-1 and BL-18 cells showed low-level expression of c-kit mRNA only detectable by subsequent Southern blot analysis (Fig 2). These two cell lines were always positive. On repeat PCR examination, the fresh MHH-BL-1 cells and the cell lines BL-2 P3HR-1, BL-16, BL-18, BL-41, BL-1 B95-8, and BL-41 P3HR-1 were only in some experiments weakly positive, indicating a very low level of expression (data not shown). c-kit mRNA was never detectable in the negative control cell line HL-6030 (data not shown). No c-kit mRNA was detected in freshly isolated T and B cells and all other BL cell lines tested (Fig 2). The question of whether c-kit mRNA was inducible and not expressed constitutively was answered by the addition of PMA or A23187 to the BL cell cultures. Only the cell line MHH-BL-1 that already expressed c-kit mRNA showed a gradual upregulation of c-kit mRNA over the time of 48 hours in the presence of A23187 (Fig 3), whereas the addition of PMA led to a rapid downregulation after 1 hour (Fig 4). c-kit transcripts in unstimulated MHH-BL-1 cells are visible in Figs 2 and 4, in contrast to Fig 3, because of the longer exposure time of the blot.

Northern blot analysis of SCF and c-kit mRNA. Northern blot analysis of SCF mRNA expression showed the presence of 5.6-kb and 3.6-kb specific transcripts in the endothelial cell line ECV 304 and a 5.0-kb c-kit specific transcript in the megakaryoblastic cell line M07e used as positive controls. SCF and c-kit mRNA were not detected in the BL cell lines tested. To investigate the possibility that SCF and c-kit mRNA expression is not constitutive but can be induced to a higher level than that found using Southern blot analysis, we stimulated the cell lines with different reagents. Figures 5 and 6 show that the addition of IL-1β, TNF-α, antihuman IgM antibodies, PMA, A23187, and SAC cells to the cell lines for 5 hours before RNA extraction did not result in the induction of SCF and c-kit mRNA.

Receptor expression on BL cells. The SCF binding was determined by chemical crosslinking of Dig-rhSCF to its receptor. The experiment was performed with the cell line MO7e used as a positive control and the cell lines MHH-BL-1 and BL-2 P3HR-1, which showed detectable c-kit mRNA expression using RT-PCR and Southern blot analysis. A cytokine-receptor complex of 165 to 170 kD was found only in MO7e cells, corresponding to the expected molecular weight of the c-kit receptor of approximately 150 kD. Specific binding of labeled SCF to its receptor could be shown by the addition of 100-fold molar excess of unlabeled SCF. BL cell lines showed no receptor-ligand complex after chemical crosslinking (Fig 7). Seven fresh BL cell preparations and the cell line MO7e were analyzed by FACS for the presence of c-kit receptors. All BL cells were gated for the CD10 antigen and found to express surface Igs. The CD117 antigen, the c-kit receptor, was found only on MO7e cells used as a positive control but on none of the BL cells (Fig 8). To determine whether A23187 treatment, which upregulates c-kit mRNA expression in MHH-BL-1 cells, would lead to the expression of c-kit receptors on the protein level, we treated MHH-BL-1 cells for 48 hours with A23187 before FACS analysis. CD117 protein remained undetectable in these stimulated cells (data not shown).

Mitogenic activity of rhSCF on BL cell lines. We tested...
rhSCF on 13 BL cell lines and used the megakaryoblastic leukemia cell line MO7e as a positive control in ³H-thymidine incorporation assays. A significant dose-dependent proliferative response to rhSCF was only detected in the MO7e cell line. None of the BL cell lines tested responded to rhSCF (Fig 9).

**DISCUSSION**

The aim of this study was to determine the role of c-kit and SCF in childhood BL. Because of the potential clinical use of SCF in patients undergoing autologous bone marrow harvest, we investigated SCF and c-kit expression in childhood BL cell lines and fresh tumor biopsy cells. We detected spontaneous specific c-kit mRNA expression on a low level in 2 of 13 BL cell lines by RT-PCR followed by Southern blot analysis. Other cell lines showed low-level expression of c-kit mRNA only with the highly sensitive RT-PCR method followed by Southern blotting and even then not in all experiments (data not shown). In only 1 of the cell lines, MHH-BL-1, a c-kit-specific PCR product was visible after 35 cycles of PCR in an ethidium bromide-stained gel. None of the BL cell lines showed hybridization to a c-kit-specific probe by Northern blot analysis with total RNA. This finding indicates absent or only very low level...
spontaneous expression of c-kit on the mRNA level in the majority of the BL cells tested.

The possibility, that c-kit is not expressed spontaneously but requires induction by other cytokines or factors present in vivo was explored by in vitro stimulation experiments. BL cells in vivo are exposed to many other factors and cytokines that theoretically could serve as costimulatory factors for SCF by inducing its receptor, especially because expression of c-kit mRNA is neither expressed nor inducible in the majority of BL cells and because no protein expression or changes in proliferation were observed on addition of rhSCF.

Some cytokines involved in normal lymphopoiesis can also be involved in the growth of B-cell malignancies. Whether SCF acts synergistically with IL-6 and IL-7 in malignant lymphopoiesis has not been investigated in detail. The autocrine stimulation by IL-6 has been shown in a small number of B-cell lymphomas. The distribution of both IL-7 receptor and c-kit receptor has been investigated in acute lymphoblastic leukemia cells. Expression of both c-kit and SCF was detected in neoplastic cells from human colon carcinoma, leading to the suggestion that the autocrine production of SCF plays a role in the growth of some human solid tumor cell lines.

BL cells appear to be derived from more mature B cells that express IgS on their surface. If they behaved like their normal counterparts, they would not be expected to express the c-kit receptor. Normal B cells that had progressed in their differentiation to light-chain rearrangement were found not to express the receptor for SCF and IL-7 anymore. Consistent with these findings in normal cells, we were not able to detect c-kit mRNA in freshly isolated normal

The autocrine stimulation by IL-6 has been shown in a small number of B-cell lymphomas. The distribution of both IL-7 receptor and c-kit receptor has been investigated in acute lymphoblastic leukemia cells. Expression of both c-kit and SCF was detected in neoplastic cells from human colon carcinoma, leading to the suggestion that the autocrine production of SCF plays a role in the growth of some human solid tumor cell lines.
B cells by 35 cycles of RT-PCR. However, it is not clear whether the process of malignant transformation leads to the persistence of less mature properties in BL cells. Support of this assumption is given by the recent findings showing detection of IL-7 receptor mRNA in BL cell lines. Moreover, whereas IL-7 receptor-positive BL cells are able to respond to rhIL-7, SCF did not act as a costimulatory factor in these cells (unpublished observation).

Fig 9. Effect of rhSCF on proliferation of BL cell lines. Cultures were maintained for 72 hours in serum-free medium in the presence of increasing amounts of SCF, pulsed for 4 hours with 0.5 μCi ³H-thymidine, and harvested for liquid scintillation counting. Data are the means and standard errors of three independent samples. The megakaryoblastic leukemia cell line MO7e was used as a positive control.
There are several observations that suggest that SCF is not mandatory for B-lineage expansion in vivo. First, in normal mice treated with a neutralizing antibody against c-kit, almost all hematopoietic progenitor cells disappeared from the bone marrow, leading to a failure to produce normal numbers of erythroid and myeloid cells, whereas B-cell lymphopoiesis was not significantly affected. Second, adult W locus anemic mice, which have functional mutations at the c-kit locus, maintain normal numbers of pre-B and B cells throughout postnatal life. These experiments show that B lymphopoiesis is possible in the absence of functional SCF or c-kit. Nevertheless, SCF can be a costimulatory factor for IL-7 in the proliferation of a subpopulation of early B cells. Another subpopulation of B-lineage cells does not require SCF and proliferates in response to IL-7 alone.

From our data we conclude that the lack of c-kit expression on the protein level in BL cells is consistent with their more mature B-cell phenotype. Also, during the process of malignant transformation, the expression of c-kit as a marker of an immature phenotype was not maintained, as apparently happened in the case of the IL-7 receptor.

Pinto et al. found that expression of c-kit receptor in human lymphomas is restricted to Hodgkin’s disease and anaplastic large-cell lymphomas (ALCL). No c-kit expression was found in lymphoid cells of reactive lymph nodes, normal tonsils, and in tumor cells from 24 cases of non-Hodgkin’s lymphoma other than ALCL.

From our studies we conclude that c-kit and SCF play no role in the process of malignant transformation and in the growth of the pediatric BL cells investigated. Based on these results, we do not expect a direct negative influence of rhSCF on chemotherapeutic treatment outcome in pediatric BL. We also think that a direct stimulation of childhood BL cells or SCF-induced direct mobilization of BL cells during an autologous peripheral stem cell harvest primed by SCF is unlikely. Secondary cytokine effects induced by the treatment of a patient with SCF, such as activation of endothelial cells and stromal elements, cannot be excluded based on our results. Whether a relevant influence on growth of the lymphoma and on treatment outcome arises from the administration of this cytokine can finally be shown only in clinical treatment studies.

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Absence of c-kit receptor and absent proliferative response to stem cell factor in childhood Burkitt's lymphoma cells

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