INTERLEUKIN-3 (IL-3) is a growth factor that supports the proliferation of many different hematopoietic cells.\textsuperscript{12} The regulatory actions of IL-3 are mediated through a plasma membrane receptor complex\textsuperscript{13} present on cells of specific hematopoietic lineages and at various stages of differentiation. FDC-P1 is an IL-3-dependent cell line derived from the bone marrow of normal DBA/2 mice.\textsuperscript{5} This cell line is representative of early hematopoietic precursor cells and grows as nonadherent cells with a colony-forming unit having granulocyte/macrophage morphology. Although FDC-P1 cells proliferate continuously in culture when IL-3 is provided, the cells are neither transformed nor tumorigenic upon injection into syngenic or nude mice.\textsuperscript{5,7} Moreover, spontaneous factor-independent cells are rarely isolated from this cell line.\textsuperscript{5,7}

Insulin and insulin-like growth factors (IGF-1 and IGF-2) are small molecular weight anabolic proteins (6 to 8 Kd) crucially involved in the use of carbohydrates as well as promoting cell growth.\textsuperscript{8,9} IGF-1 stimulates both acute and long-term metabolic processes and is mainly synthesized in the liver under the control of growth hormone.\textsuperscript{8,10} IGF-1 is present in serum at varying concentrations (10 to 100 nmol/L) and is complexed with binding proteins that serve to limit its biologic effects.\textsuperscript{8,9} IGF-1 was initially thought to have only endocrine actions; however, recently IGF-1 has been shown to have autocrine and paracrine functions and may be involved in the malignant transformation of certain cells.\textsuperscript{8,10,14}

Insulin and IGF-1 exert their biologic effects by binding to their cognate receptors (IN-R, IGF-1R).\textsuperscript{8,15} IN-R and IGF-1R are similar in structure and the genes encoding both receptors probably evolved from a common progenitor gene.\textsuperscript{8,16} Although insulin and IGF-1 can bind each other's receptor, approximately 100-fold more ligand is required to activate the heterologous receptor.\textsuperscript{8,17} The receptors for IGF-1 or insulin, factor-independent cells were rarely isolated. Growth of these cells was also inhibited by the αr-3 MoAb and they expressed 100- to 400-fold more IGF-1 receptors than uninfected FDC-P1 cells. The endogenous IGF-1 and/or insulin present in the calf serum may have enabled their growth because these cells, unlike the parental cells, would proliferate in serum-free defined media and their growth was again inhibited by the αr-3 MoAb. These results demonstrate that IGF-1 can replace IL-3 for growth when FDC-P1 cells overexpress the IGF-1R. Given the fairly ubiquitous expression of the IGF-1 receptor, these and additional experiments might help to determine whether increased expression of endogenous receptors by cells can lead to leukemogenesis and tumorigenesis. Moreover, hIGF-1R–infected cells will be useful in investigating the mechanisms of IGF-1-mediated signal transduction because they are now known to proliferate in response to IGF-1.
generation of the viral oncogenes v-erb-B, v-kit, and v-fms, which are related in structure to growth factor receptors.22-24

After infection of IL-3–dependent cell lines with retroviral constructs containing the IL-3 gene, factor-independent cell lines have been isolated. These cells were transformed by an autocrine mechanism.25-27 Viral oncogenes with tyrosine kinase activity (v-abl, v-src, v-fms, and v-erb-B) abrogated the growth factor-dependency of certain hematopoietic cells by a non-autocrine mechanism, because the transformed cells grew in a density-independent fashion and growth factors supporting autocrine growth were not detected.28-31 Studies with temperature-sensitive v-abl mutants demonstrated that expression of the virally encoded tyrosine kinase was necessary for growth factor-independence.32,33 These investigations as well as others aimed at elucidating IL-3–mediated signal transduction34-36 have implicated tyrosine kinases in the regulation of IL-3–induced cellular proliferation.

Recombinant retroviruses containing the proto-oncogenes c-erb-B or c-fms abrogated IL-3 dependency of factor-dependent cell lines providing exogenous epidermal growth factor (EGF) or macrophage colony-stimulating factor (M-CSF) were added, respectively.40,42 Although in one study, M-CSF–independent cells were isolated after c-fms infection and their growth was inhibited upon addition of anti–M-CSF-R antibodies.43 In addition, the human IGF-1R malignantly transformed NIH-3T3 cells when either IGF-1 or supraphysiological concentrations of insulin were provided in the culture medium.44 These cytokine receptor genes are able to transform cells by ligand-dependent and -independent mechanisms and represent unique opportunities to investigate receptor-mediated signal transduction and transformation. To determine whether overexpression of the IGF-1R would relieve the IL-3 dependency of hematopoietic cells, FDC-P1 cells were infected with a retroviral construct containing the human IGF-1R. The infected cells overexpressed the IGF-1R and were no longer dependent on IL-3 for cell growth. While the vast majority (>99%) of transformed cells required exogenous ligand (IGF-1) for growth, a minority of cells proliferated in the absence of exogenously supplied ligand and increased IGF-1 receptor expression was correlated with their growth.

MATERIALS AND METHODS

Cell culture. Cells were maintained in a humidified 5% CO₂ incubator with Dulbecco's modified Eagle's medium (DMEM) containing 5% iron-supplemented bovine calf serum (CS; HyClone, Logan, UT). This CS contained approximately 150 pmol/L insulin, which in our culture conditions (DMEM + 5% CS) would correspond to 7.5 pmol/L. DMEM containing 5% CS will be referred to as medium throughout. The IL-3–dependent myeloid line, FDC-P1, was maintained in medium supplemented with clarified supernatant (10%) prepared from the WEHI-3B (D') cell line as a source of IL-3. rIGF-1 was generously provided by Eli Lilly Co (Indianapolis, IN). Insulin, genetecin (G418), and insulin-transferrin-sodium selenite media supplement (ITES) were purchased from Sigma (St Louis, MO). Cellular proliferation assays were performed with [³H]thymidine (6.7 Ci/mmol, New England Nuclear [NEN], Boston, MA), as described.44 Ornithine decarboxylase (ODC) activity was determined in cellular extracts with [¹⁴C]-ornithine (51.6 mCi/mmol; Amersham, Arlington Heights, IL), as described.45 IL-3– or insulin-deprivation experiments were performed as described.46-48 Cell concentrations were determined with a Coulter Counter (Hialeah, FL). Growth curve experiments were performed with exponentially growing cells that were washed twice with phosphate-buffered saline (PBS), resuspended at 2 × 10⁶ cells/mL in 10 mL of medium with respective supplements, and then plated in 25-cm² T-flasks (Corning, Corning, NY). Growth curve experiments in the presence of the hIGF-1R (aI-R3) monoclonal antibody (MoAb),49 were performed as above, except serial dilutions of the MoAb were included and the cells were plated in 4 mL of medium to conserve MoAb. The aI-R3 MoAb was provided as an ascites fluid by Dr Judson J. Van Wyk (University of North Carolina, Chapel Hill, NC) or was obtained from Oncogene Sciences (Manhasset, NY). Fluorescence-activated cell sorter (FACS) analysis was performed with a 1:250 dilution of the MoAb on a Becton Dickinson FACS 440 (Becton Dickinson, Mountain View, CA), as described.43,44

Retroviral infection of cells. Viral supernatants were harvested from Psi2 and PA317 cells that produce the LINS and LNL6 viral constructs, respectively.43 Viral supernatants were harvested by centrifugation and filtration through a 0.45-μm filter. Viral titers (focus forming units/mL) were determined as the number of G418 resistant (G418') colonies on NIH-3T3 cells. FDC-P1 cells (5 × 10⁴) were infected with serial dilutions of the viral stocks in 10 mL of medium containing IL-3 and 10 μg/mL polybrene (Sigma). Mock-infected cells were treated as virally infected cells, except medium was added in place of the viral stock. After 4 hours of incubation, 40 mL of medium containing IL-3 was added to the cultures to dilute the concentration of polybrene. Twenty-four hours after infection, the cells were washed twice with PBS and then plated under the various selective conditions. Selective medium for inheritance and selection of neo was G418 (2 mg/mL) in the presence of IL-3 (10% WEHI-3B supernatant). Selective media for activation of the hIGF-1R and abrogation of IL-3 dependency were medium containing 50 nmol/L IGF-1, 10 nmol/L IGF-1, or 1 μmol/L insulin. Finally, cells were plated in medium lacking additional supplements to determine whether IL-3-independent cells could be isolated in the absence of exogenously supplied IGF-1 and insulin. Cells (2 × 10⁶ cells/well) were plated in 96-well flat bottom plates (Corning) and every 3 days fresh medium was added. Foci of growing cells were visible 2 to 6 days after infection and after approximately 10 to 14 days, the cells were expanded into 1 mL cultures (24-well plates, Corning) in the respective selective medium. Subsequently, these wells were expanded into 10 mL and then 50 mL cultures. Essentially all wells growing in the 96-well plates could be expanded readily into mass cultures. Individual clones were subsequently isolated by limiting dilution in 96-well plates (round bottom) in medium containing the appropriate selective agents. The nomenclature of the infected cells is FD/ followed by LINS and LNL6 for FDC-P1 cells infected by either the LINS or LNL6 virus respectively. Parentheses after LINS or LNL6 indicate the selection conditions used to isolate the cells and C indicates that the cells were cloned by limiting dilution.

IGF-1 binding assay. Log phase cells were collected by centrifugation, washed twice with PBS, and resuspended in Kreb's ringer buffer (KRB) containing 1% bovine serum albumin (Sigma). Cells (1 × 10⁶) were added to sterile polypropylene tubes (Falcon, Lincoln Park, NJ; 12 × 17 mm) followed by the addition of serial dilutions of cold IGF-1 (10⁻⁸ to 10⁻¹⁰ mol/L) and 100,000 cpm of [¹²⁵I]-IGF-1 (10⁻¹⁹ mol/L, 280 μCi/μg, generously provided by Eli Lilly Co). The binding mixture was vortexed and incubated at 4°C for 18 hours. The tubes were then centrifuged and the pellets washed twice with KRB and then counted on a Beckman Gamma
ABROGATION OF IL-3-DEPENDENCY BY IGF-1R

5500 counter. The specific $^{125}$I bound was determined by subtraction of the cpm observed with cold competitor (1 μmol/L) from the cpm observed with no competitor. Each data point in the IGF-1 binding studies was performed in triplicate and the standard error of the mean was less than 5%.

RNA analysis. Total RNAs (20 μg) were denatured with formaldehyde/formamide and electrophoresed in 1.2% agarose gels containing formaldehyde. Blotting of RNA was performed with Gene Screen Plus nylon membranes (NEN) as described. Filters were prehybridized, hybridized, and washed as described. Hybridization was performed with $10^5$ cpm/mL of probe prepared by random oligonucleotide primer extension. The sizes of mRNA encoding different genes were determined by linear regression analysis with the mobilities of either an ethidium bromide-stained 0.24- to 9.5-kb RNA ladder (BRL, Gaithersburg, MD) or ribosomal RNA serving as internal references. The c-myec exon 2 and 3 probe was obtained from Dr Michael Cole (Princeton University, Princeton, NJ). The ODC probe was provided by Dr Carolyn Steglich (East Carolina University, NC). The β-actin probe (p7000) was obtained from Oncor (Gaithersburg, MD).

RESULTS

FDC-P1 is an IGF-1-responsive cell line. We investigated the ability of recombinant (r) IGF-1 to stimulate $^3$H-thymidine incorporation upon addition to FDC-P1 cells. As shown in Fig 1A, IGF-1-induced $^3$H-thymidine incorporation over the background level that was observed with no growth factor addition. From these experiments, it was apparent that IGF-1 stimulated $^3$H-thymidine incorporation at physiologic (10 to 100 nmol/L) concentrations. The maximal level of $^3$H-thymidine incorporation obtained with IGF-1 was approximately threefold lower than that observed with IL-3 (125,000 to 150,000 cpm), at IL-3 concentrations (10% to 20% WEHI-3B-conditioned supernatant) normally used for the propagation of this cell line. To determine whether addition of IGF-1 and IL-3 would result in an additive or synergistic response, suboptimal (constant) doses of IL-3 were added with different concentrations of IGF-1. As observed in Fig 1A, a synergistic effect was observed, suggesting that IL-3 and IGF-1 induced overlapping pathways of signal transduction, although addition studies are necessary to determine the protein substrates phosphorylated by IL-3 and IGF-1. Similar results were seen with IL-3 obtained both from WEHI-3B supernatants and rIL-3 (data not presented).

To determine whether IGF-1 induced an additional biochemical marker associated with cellular proliferation, we examined ODC activity after IGF-1 addition to IL-3-deprived FDC-P1 cells. ODC is the rate-limiting enzyme of polyamine synthesis and is necessary for cell growth. We chose to use 10 nmol/L IGF-1 in these experiments, because this concentration is physiologic and induced nearly as much $^3$H-thymidine incorporation as higher, non-physiologic doses. Both IGF-1 and IL-3 stimulated ODC activity, whereas very low ODC expression was observed in cells that did not receive either growth factor (Fig 1B). Again, as observed in $^3$H-thymidine incorporation experiments, the ODC activity detected after IGF-1 addition was lower than that obtained with IL-3.

Although IGF-1 induced $^3$H-thymidine incorporation and ODC in FDC-P1 cells, IGF-1 did not replace IL-3 for continuous growth (Fig 1C). Higher concentrations of IGF-1 (50, 100, and 500 nmol/L IGF-1) were similarly examined and cellular proliferation was not observed in growth curve assays, nor were cell lines recovered from extended culture periods that would grow in the presence of only IGF-1 (data not presented).
Infection of IL-3-dependent cells with a retrovirus containing the human IGF-1R gene. To investigate further the effects of IGF-1 on hematopoietic cells and to determine whether overexpression of the human IGF-1R (hIGF-1R) in the presence of either IGF-1 or insulin would abrogate IL-3 dependency, FDC-P1 cells were infected with a recombinant retrovirus (LISN) containing the hIGF-1R gene and the dominant selectable marker neo, which confers resistance to the antibiotic G418. The expression of the hIGF-1R in the infected cells is driven by the retroviral long terminal repeat sequences (LTR), resulting in constitutive high level expression. Cells were also infected with a control virus (LNL6) that contains just the neo gene.

Twenty-four hours after infection of FDC-P1 cells with the hIGF-1R virus (LISN), the neo control virus (LNL6), or a mock infection, the pools of cells were divided five ways and incubated in medium (DMEM plus 5% CS) with the following supplements: (1) G418 and IL-3 for selection of neo (G418 colonies); (2) 50 nmol/L or 10 nmol/L rIGF-1, to determine whether activation of the introduced hIGF-1R would relieve IL-3 dependency; (3) 1 μmol/L insulin to determine whether supraphysiologic concentrations of insulin would abrogate IL-3 dependency; or (4) no supplements, to determine whether IL-3-independent cells could be isolated in the absence of exogenously added IGF-1 and insulin. We have used supraphysiologic concentrations of insulin (1 μmol/L) that activate the IGF-1Rs in place of rIGF-1 in some experiments (as indicated) due to economical constraints.

As indicated in Table 1, G418' cell growth was observed after infection of FDC-P1 with either the hIGF-1R or the control virus. Moreover, as expected, no G418' growth was observed in the mock-infected cultures. However, colonies growing in medium containing either IGF-1 or insulin but lacking IL-3 were only observed after infection with the hIGF-1R (LISN) retrovirus.

Table 1. Infection of FDC-P1 Cells With an hIGF-1R-Containing Retrovirus

| Virus | Introduced Genes | Selection Conditions | Media Supplement | No. of Wells Positive for Growth | (%)
<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>LISN</td>
<td>hIGF-1R</td>
<td>IL-3</td>
<td></td>
<td>288/288*</td>
<td>(100)</td>
</tr>
<tr>
<td></td>
<td>+ neo</td>
<td>G148 (50 nmol/L)†</td>
<td></td>
<td>96/96</td>
<td>(100)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Insulin</td>
<td></td>
<td>181/192</td>
<td>(84)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>None</td>
<td></td>
<td>9/192</td>
<td>(3)</td>
</tr>
<tr>
<td>LNL6†</td>
<td>neo</td>
<td>G148 + IL-3</td>
<td></td>
<td>192/192</td>
<td>(100)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IGF-1 (50 nmol/L)</td>
<td></td>
<td>0/96</td>
<td>(0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Insulin</td>
<td></td>
<td>0/192</td>
<td>(0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>None</td>
<td></td>
<td>0/192</td>
<td>(0)</td>
</tr>
<tr>
<td>Mock</td>
<td>None</td>
<td>G148 + IL-3</td>
<td></td>
<td>0/96</td>
<td>(0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IGF-1 (50 nmol/L)</td>
<td></td>
<td>0/96</td>
<td>(0)</td>
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<td></td>
<td></td>
<td>Insulin</td>
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<td>(0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>None</td>
<td></td>
<td>0/96</td>
<td>(0)</td>
</tr>
</tbody>
</table>

Infection and selection conditions are described in Materials and Methods.

*It was observed that there were multiple transformed cells arising in many wells.
†Similar results were observed with 10 nmol/L IGF-1.
‡This viral construct transfers neo only.

The relative efficiency of transformation to IL-3 independence cannot be determined from the data presented in Table 1, because many of the wells arising after infection with hIGF1-R contained multiple foci of growing cells. Therefore, we infected FDC-P1 cells with serial dilutions of hIGF-1R (LISN) and again divided the infected cultures five ways. As indicated in Fig 2A, threefold to 10-fold more G418' wells were observed than when the same cultures were plated in either IGF-1 or insulin at viral dilutions that yielded 50% of the wells positive for cell growth. Therefore, although expression of the hIGF-1R enabled cells to grow in...
the presence of IGF-1 or insulin, abrogation of IL-3 dependency was not as efficient as transformation to G418. When hIGFIR-infected cells were cultured in medium lacking IL-3, IGF-1, or insulin, a 100- to 1,000-fold lower frequency of IL-3-independent colonies was observed after culture of hIGFIR-infected cells in medium lacking IL-3, IGF-1, or insulin. These colonies were only observed after infection with the LISN virus and not after either mock or control virus infections.

**Plating efficiency and growth properties of hIGFIR-infected cells.** To determine whether the cells infected with hIGF-1R and selected for G418 readily gave rise to IL-3-independent cells (IGF-1/insulin–dependent cells), limiting dilution analysis was performed (Fig 2B). When either the parental- (uninfected cells) or the control virus- (LNL6) infected cells were plated in IL-3, the cloning efficiency was close to 1 (Fig 2B1 and B2). However, no colonies were observed when these cells were plated in the absence of IL-3.

In contrast, when hIGFIR-infected FDC-P1 clones (selected in G418 and IL-3 and cloned by limiting dilution) were cultured in medium containing either IGF-1 or insulin, IGF-1/insulin–independent cells were readily recovered, however, at approximately threefold to 10-fold lower levels than in medium containing IL-3 (Fig 2B3 and B4). Thus, each hIGFIR-infected cell could readily give rise to IGF-1/insulin–dependent cells.

When the hIGFIR-infected cells were plated in medium containing no additional supplements, one of two clones gave rise, at a very low frequency, to cells growing in the absence of IL-3, IGF-1, or insulin (Fig 2B3 and B4). Similar results were observed with seven other clones examined. Therefore, while factor-independent cells could be recovered from some clonal cell lines that were infected with the hIGF-1R virus, they were not isolated from uninfected or the neo control virus-infected cells.

When uninfected or control virus– (LNL6) infected FDC-P1 cells were grown in IL-3 they divided approximately every 24 hours, whereas upon culture in medium lacking IL-3 they did not proliferate (Fig 1C). However, when hIGFIR-infected (either G418 or IGF-1 selected) cells were cultured in IL-3 or IGF-1, they doubled approximately every 24 hours (Fig 3A and B). Moreover, growth of these hIGFIR-infected cells was dependent on the concentration of IGF-1 provided. Growth was observed at IGF-1 concentrations ranging from 5 to 50 nmol/L and was not observed at 0.5 or 0.05 nmol/L IGF-1 (Fig 3A and B). Similar results were observed with the hIGFIR-infected cells selected in insulin (data not presented). Therefore, these IL-3–independent cells grew in response to IGF-1 or insulin in a ligand-dependent fashion.

In contrast, addition of either IGF-1 or IL-3 was not necessary for the growth of hIGFIR-infected cells that were isolated in medium containing no supplements. Addition of exogenous IGF-1, insulin, or IL-3 did not reproducibly enhance cellular proliferation (Fig 3C and F).

**MoAbs to hIGF-1R inhibit the growth of hIGFIR-infected cells.** To determine whether the cellular growth was mediated by the introduced hIGF-1R in LISN-infected cells, we included the hIGF-1R MoAb (aIR-3) in the culture conditions. The aIR-3 MoAb attaches at the ligand binding site, preventing IGF-1 binding, thereby blocking receptor activation. 18,20,43,47,51-54 The aIR-3 MoAb inhibited the growth of the hIGFIR-infected cells isolated after IGF-1 selection when they were cultured in IGF-1 (Fig 3E). However, when the same cells were cultured in IL-3, the aIR-3 MoAb did not inhibit cell growth, indicating that the cells could still respond to the growth-promoting effects of IL-3 (Fig 3D).

**Fig 3.** Ligand-dependent growth of hIGFIR-infected FDC-P1 cells. Cells were cultured in the presence of medium (DMEM + CS) containing the following supplements: IL-3 (□), IL-3 + aIR-3 antibody (●), 50 nmol/L IGF-1 (○), 50 nmol/L IGF-1 + aIR-3 (♦), 10 nmol/L IGF-1 (△), 10 nmol/L IGF-1 + aIR-3 (●), 5 nmol LIGF-1 (+), 0.5 nmol/L IGF-1 (X), and no addition (△). (A) FD/LISN/G418C2, (B) FD/LISN/IGF-1C1, (C) FD/LISN(DMEM)C3, (D) FD/LISN/IGF-1C1, (E) FD/LISN/IGF-1C1, (F) FD/LISN(DMEM)C1. The concentration of aIR-3 was a 1:400 dilution of ascites fluid in these experiments. These experiments were repeated with other LISN-infected clones five times and similar results were observed.
Moreover, the aIR-3 MoAb inhibited "H-thymidine incorporation when the hIGF1R-infected cells were cultured in IGF-1 but not when they were cultured in IL-3 (data not presented). Therefore, an unblocked hIGF-1R on the cell surface was necessary for the IGF-1-induced growth.

In addition, the aIR-3 MoAb inhibited the growth of the hIGF1R-infected cells selected in medium lacking additional supplements (Fig 3F), suggesting that the growth of these cells was stimulated by the IGF-1 and/or insulin present in the CS.

IGF-1 concentrations in CS are reported to be at least 4 nmol/L. An estimate of the IGF-1 concentration present in the CS used in these experiments (5% CS) would be 0.2 nmol/L. This concentration of IGF-1 stimulated "H-thymidine incorporation in hIGF1R-infected but not in uninfected FDC-P1 cells (Fig 4). In parallel titration experiments, the responses of hIGF1R-infected and uninfected FDC-P1 cells to IL-3 were similar (data not presented). Therefore, hIGF1R-infected cells responded to lower IGF-1 concentrations than uninfected FDC-P1 cells.

Additional experiments indicated that the hIGF1R-infected cells, which were isolated in the absence of medium supplements, proliferated in serum-free ITES medium. Moreover, their growth in this medium was inhibited by the aIR-3 MoAb (data not presented). Therefore, hIGF1R-infected cells responded to lower levels of IGF-1 receptor expression. To determine the levels of IGF-1R present on uninfected and hIGF1R-infected cells, radiolabeled ligand ([125I]-IGF-1) binding experiments were performed. Scatchard analysis indicated that the hIGF1R-infected cells (G418, IGF-1, or insulin selected) contained 20- to 200-fold more IGF-1 receptors than the parental, control virus-infected or IL-3-independent cells that arose after Abelson murine leukemia virus (A-MLV) infection (Table 2).

The hIGF1R-infected clones that were isolated after growth in medium without additional supplements displayed the highest levels of IGF-1R, 100 to 400 more than detected on uninfected FDC-P1 cells. Therefore, IL-3-independent cell growth was associated with high levels of IGF-1 receptor expression.

Detection of the hIGF-1R on the cell surface of infected cells by FACS analysis. To determine the presence of hIGF-1R on the cell surface, we performed FACS analysis with the aIR-3 MoAb. This MoAb does not recognize the murine IGF-1R. The hIGF-1R was detected on the cell surface of LISN-infected cells but not on the surface of uninfected or control virus-infected cells. Consistent with the [125I]-ligand binding experiments, the hIGF1R-infected cells that were selected in medium alone displayed more hIGF-1R on the cell surface than other hIGF1R-infected cells (data not presented).

Lack of autocrine growth factor production in hIGF1R-infected cells. To determine whether the cells transformed to IL-3 independence released growth factors that would support autocrine growth, we assayed supernatants and mRNA preparations from hIGF1R-infected cells for cytokine expression. No cell stimulatory activity was detected in the supernatants that would support the growth of FDC-P1 (data not presented). Moreover, mRNA transcripts encoding growth factors (IL-3, IGF-1, and granulocyte-macrophage CSF [GM-CSF]) were not detected (by Northern

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**Table 2. Quantitation of IGF-1 Receptors in FDC-P1 and hIGF1R-Infected Cells**

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Receptor Cells*</th>
<th>Fold Above FDC-P1</th>
</tr>
</thead>
<tbody>
<tr>
<td>FDC-P1</td>
<td>8.4 ± 10^5</td>
<td>—</td>
</tr>
<tr>
<td>FD-abl-1†</td>
<td>9.6 ± 10^5</td>
<td>—</td>
</tr>
<tr>
<td>FD/LISN/DMEM/C.4</td>
<td>3.0 ± 10^5</td>
<td>35x</td>
</tr>
<tr>
<td>FD/LISN/DMEM/C.3</td>
<td>3.1 ± 10^5</td>
<td>37x</td>
</tr>
<tr>
<td>FD/LISN/DMEM/C.2</td>
<td>4.2 ± 10^5</td>
<td>50x</td>
</tr>
<tr>
<td>FD/LISN/DMEM/C.1</td>
<td>7.9 ± 10^5</td>
<td>94x</td>
</tr>
<tr>
<td>FD/LISN/DMEM/C.1</td>
<td>7.9 ± 10^5</td>
<td>94x</td>
</tr>
<tr>
<td>FD/LISN/DMEM/C.2</td>
<td>1.1 ± 10^5</td>
<td>130x</td>
</tr>
<tr>
<td>FD/LISN/DMEM/C.3</td>
<td>3.4 ± 10^6</td>
<td>405x</td>
</tr>
<tr>
<td>FD/LISN/DMEM/C.4</td>
<td>3.1 ± 10^6</td>
<td>369x</td>
</tr>
<tr>
<td>FD/LISN/DMEM/C.5</td>
<td>3.5 ± 10^5</td>
<td>416x</td>
</tr>
<tr>
<td>FD/LISN/DMEM/C.6</td>
<td>3.5 ± 10^6</td>
<td>416x</td>
</tr>
</tbody>
</table>

*Table 2, 125I-IGF-1 binding conditions are described in Materials and Methods.
†Determined by the X intercept on Scatchard analysis.
‡FD-abl-1 is an A-MLV-infected FDC-P1 factor-independent cell line. Parentheses indicate the selection conditions used to derive the cell line.
ABROGATION OF IL-3-DEPENDENCY BY IGF-1R

***IGF-1 stimulates c-myc and ODC expression in hIGF1R-infected cells.*** IL-3 induces the expression of the c-myc and ODC genes in FDC-P1 cells\(^{1,2,4,5,5}\) and these genes have been associated with cellular proliferation. To determine whether IGF-1 stimulated these genes in hIGF1R-infected (ligand-dependent) cells, we added either IGF-1 or IL-3 to factor-deprived cells. As observed in Fig 5, both IGF-1 and IL-3 stimulated c-myc and ODC expression in hIGF1R-infected cells in a concentration-dependent fashion. mRNA levels of transcripts encoding c-myc and ODC in uninfected factor-deprived FDC-P1 cells exposed to IGF-1 were much lower and difficult to reproducibly detect (data not presented). Thus, IGF-1 stimulated the expression of genes associated with cellular growth in ligand-dependent hIGF1R-infected cells.

**DISCUSSION**

IGF-1 is a physiologically important growth factor involved in normal body development by stimulating the growth of cells of diverse lineages.\(^6,10\) Moreover, IGF-1 affects hematopoiesis, because it can promote erythroid colony formation.\(^6\) Although IGF-1 was initially considered an endocrine growth factor, recent investigations have shown that this cytokine has both autocrine and paracrine roles and, under certain circumstances, IGF-1 has been implicated in cellular transformation.\(^61,6b\) Recently, it has been demonstrated that the IGF-1 receptor can function as an oncogene when overexpressed in the presence of ligand.\(^6b\) Therefore, deregulation of this receptor-ligand interaction can lead to malignancy.

In this study, we examined the effects of IGF-1 on hematopoietic cells to further understand the mechanisms by which growth factors regulate cellular proliferation. Specifically, we were interested in determining whether the tyrosine kinase activity stimulated by IGF-1 would substitute for the activity normally induced by IL-3. Although FDC-P1 cells responded to IGF-1 at physiologic concentrations, IGF-1 did not replace IL-3 for continuous growth of uninfected FDC-P1 cells. Consistent with these observations, the magnitude of \(^\text{H}\)-thymidine incorporation and ODC activity observed with IGF-1 were lower than that obtained with IL-3.

When FDC-P1 cells were infected with a recombinant retrovirus containing the human IGF1R gene, which enabled the cells to overexpress the IGF-1 receptor, IL-3-independent cells were readily isolated, providing IGF-1 or supraphysiologic concentrations of insulin were provided in the culture medium. The growth of these cells was dependent on the presence of IGF-1 and cytokines supporting autocrine growth of FDC-P1 cells were not detected. The infected cells displayed the human IGF-1R on the cell surface and MoAb to the hIGF-1R inhibited IGF-1- but not IL-3-induced growth. Therefore, in the presence of ligand and high levels of receptor, the cells were no longer dependent on IL-3 for growth and were transformed to factor independence by a ligand-dependent mechanism.

We were concerned that additional genetic changes were required for the growth of the hIGF1R-infected cells in absence of IL-3, because such events have been proposed necessary for abrogation of IL-3 dependency after infection with retroviruses containing certain viral oncogenes (eg, \textit{myc, src, fms}\(^s\)).\(^2,4,5,5,5\) However, limiting dilution experiments indicated that each G418-selected clone readily gave rise to cells that grew in response to either IGF-1 or insulin. The difference in subcloning efficiency (threefold to 10-fold) was probably a reflection that the cells grew better in microtiter wells in the presence of IL-3 than either IGF-1 or insulin, because when IGF-1-selected cells were similarly examined, they also had a higher subcloning efficiency in IL-3. The high frequency of IL-3-independent (IGF-1-insulin-dependent) cells suggested that additional genetic mutations were not necessary for IGF-1-induced growth. Moreover, subclones derived from G418-selected clones that were isolated in medium containing either IGF-1 or insulin contained the same proviral copy number and integration sites as determined by Southern blotting (data not presented). Together, these experiments suggest that overexpression of a unrearranged hIGF1R was sufficient for the continuous growth of the cells.
for growth of FDC-P1 cells in the presence of IGF-1 or insulin.

A 100- to 1,000-fold lower frequency of IL-3-independ-
ent cells was recovered when hIGFIR-infected cells were
grown in medium lacking exogenous added IGF-1. This
frequency of IL-3 independence is 10^{-7} to
10^{-8}. Moreover, these cells displayed the highest
levels of hIGF-1R, they proliferated in serum-free defined
medium, and again their growth was inhibited when antibo-
dies to the IGF-1 receptor were included in the culture
medium. Therefore, it is likely that the cells that were
isolated in the absence of media supplements proliferated
in response to the IGF-1 and/or insulin contained in CS.

A conceivable reason why uninfected FDC-P1 cells did
not grow in response to IGF-1 was the low number of
IGF-1R molecules present on their cell surface. Transfor-
mation to IL-3 independence was always associated with
high levels of IGF-1R expression. Normal cells may tightly
regulate IGF-1 receptor expression because uncontrolled
expression could lead to transformation by paracrine or
autocrine mechanisms.

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ABROGATION OF IL-3-DENSITY BY IGF-1R


Growth-promoting effects of insulin-like growth factor-1 (IGF-1) on hematopoietic cells: overexpression of introduced IGF-1 receptor abrogates interleukin-3 dependency of murine factor-dependent cells by a ligand-dependent mechanism

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