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Interleukin 7 receptor ligation stimulates tyrosine phosphorylation, inositol phospholipid turnover, and clonal proliferation of human B-cell precursors

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ABSTRACT **Functional interleukin 7 (IL-7) receptors are expressed on the surface of multiphenotypic, biphenotypic, and immature B-lineage human lymphoid precursor cells with germ-line immunoglobulin heavy-chain genes but not on more mature B-lineage lymphoid cells with rearranged and/or expressed immunoglobulin heavy-chain genes. Thus, IL-7 may have an important regulatory role during the earliest stages of human B-cell ontogeny. The engagement of the surface IL-7 receptors on immature B-cell precursor cells with recombinant human IL-7 (rhIL-7) results in enhanced tyrosine phosphorylation of multiple phosphoproteins, stimulates inositol phospholipid turnover and DNA synthesis, and promotes their clonal proliferation. These effects are (*i*) specific for rhIL-7, since rhIL-3, rhIL-4, rhIL-5, rhIL-6, and recombinant human granulocyte colony-stimulating factor do not elicit similar activities on IL-7 receptor-positive human pro-B cells; and (*ii*) mediated by IL-7 receptors, since they are not observed in IL-7 receptor-negative B-lineage lymphoid cell populations. rhIL-7-induced tyrosine phosphorylation on the 35-, 53-, 55-, 62-, 69-, 76-, 94-, 150-, 170-, and 190-kDa substrates as well as rhIL-7-induced stimulation of inositol phospholipid turnover are abrogated by the tyrosine kinase inhibitor genistein. These results demonstrate that the IL-7 receptor on immature human B-cell precursor populations is intimately linked to a functional tyrosine kinase pathway and tyrosine phosphorylation is an important and perhaps mandatory step in the generation of the IL-7 receptor-linked transmembrane signal.**

Human B-cell development, activation, proliferation, and differentiation are regulated at multiple levels by a complex interplay of specific cell types and soluble growth factors (1–10). Although regulation of the late steps in human B-cell ontogeny has become better understood, the growth regulatory signals governing the proliferation and differentiation of B-cell precursors (BCPs) during the early stages in human B-lineage lymphopoiesis remain unclear (1–3).

Interleukin 7 (IL-7) is a stromal cell-derived soluble growth factor with lymphoid precursor cell (LPC) growth-promoting activity (4, 5). Recently, a cDNA encoding murine IL-7 has been cloned and recombinant IL-7 has been shown to promote the proliferation of murine pre-B, pro-B, T-cell precursor as well as mature T cells (6–11). The murine IL-7 cDNA was subsequently used to probe a human genomic library, and a cDNA clone encoding biologically active human IL-7 protein was isolated by cross-species hybridization with the homologous murine clone (12, 13). In this report, we examine the expression and function of IL-7 receptors (IL-7Rs) at distinct stages of differentiation in human B-lineage lymphopoiesis as well as the biochemical nature of IL-7-mediated transmembrane signal. We show that engagement of high-affinity IL-7Rs with recombinant human IL-7 (rhIL-7) stimulates tyrosine phosphorylation, inositol phospholipid turnover, and clonal proliferation of human B-cell precursors at the earliest stages of human B-cell ontogeny.

MATERIALS AND METHODS

Human LPCs. The isolation and purification of immunophenotypically distinct LPC populations from various sources were performed as described (14–16). Flow cytometric analyses and cell sorting were performed according to published procedures on a FACStar Plus flow cytometer (Becton Dickinson) (14–16). All human tissue specimens were used following the guidelines of the University of Minnesota Committee on the Use of Human Subjects in Research for secondary use of pathologic or surgical tissue.

Hematopoietic Growth Factors. rhIL-7 (2.8×10^6 units/mg) was a generous gift of S. Gillis (Immunex, Seattle) and C. R. Faltynek (Sterling Drug, Malvern, PA). rhIL-7 was cloned, expressed, and purified to homogeneity as reported in detail elsewhere (12, 13). Purified rhIL-3 (2.5×10^6 units/mg), purified rhIL-6 (4×10^6 units/mg), and rhIL-5 (crude supernatant from COS cells transfected with an expression plasmid containing the human IL-5 cDNA; 5×10^3 units/ml) were kindly provided by S. Clark (Genetics Institute, Cambridge, MA). Recombinant human granulocyte colony-stimulating factor (rhG-CSF) (1×10^8 units/mg) was kindly provided by L. Souza (Amgen Biologicals).

Preparation and Characterization of ^{125}I-Labeled rhIL-7 (^{125}I-rhIL-7). Biologically active ^{125}I-rhIL-7 (8×10^{10} cpm/mg) was prepared from purified rhIL-7 by a solid-phase iodination technique with Iodo-Beads (Pierce), following the specific recommendations of the manufacturer. Equilibrium binding assays and Scatchard analyses with ^{125}I-rhIL-7 were performed in the presence and absence of 1000-fold excess unlabeled rhIL-7 to characterize IL-7Rs as described (15, 16).

Colony Assays. Human lymphoid precursor cells were cultured in the presence of hematopoietic growth factors at the indicated concentrations for 7 days and were assayed for colony formation as described (17–19).

Analysis of Stimulation of Inositol Phospholipid Turnover in Human Lymphoid Precursor Cells. Cells were washed twice in RPMI 1640 medium/2.5% (vol/vol) bovine calf serum and were resuspended at 1×10^7 cells per ml in inositol-free

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Abbreviations: BCP, B-cell precursor; IL-7, interleukin 7; LPC, lymphoid precursor cell; rhIL-7, recombinant human IL-7; IL-7R, IL-7 receptor; rhG-CSF, recombinant human granulocyte colony-stimulating factor; [^{3}H]Ins, *myo*-[1,2-^{3}H(N)]inositol; InsP_3, inositol 1,4,5-trisphosphate; PMA, phorbol 12-myristate 13-acetate; PI-PLC, phosphatidylinositol phospholipase C.

Dulbecco's modified Eagle's medium (GIBCO). Cells were incubated with 50 μCi of *myo*-[1,2-^{3}H(N)]inositol ([^{3}H]Ins) per ml (66.1 Ci/mmol; 1 Ci = 37 GBq; New England Nuclear) for 4 hr at 37°C in 5% CO_2/95% air. Subsequently, cells were centrifuged at 300 × *g* for 5 min, washed once in minimal essential medium (MEM), and resuspended in MEM at a concentration of 2.5 × 10^6 cells per ml. Duplicate samples of [^{3}H]Ins-labeled cells (6 × 10^5 cells per 245 μl per tube) received either 5 μl of MEM or 5 μl of rhIL-7 to a final concentration of 10 ng/ml. The cells were incubated at 37°C with constant agitation for 30 sec, 1 min, or 10 min. Incubations were terminated and samples were extracted with 1.1 M ice-cold perchloric acid as described (20, 21). The extracted [^{3}H]Ins phosphates were resolved by high-performance liquid chromatography (HPLC) on a 4.6 mm × 25 cm Ultrasil strong anion exchange (SAX) column (Beckman) with a linear gradient of 0.001–1 M $NH_4H_2PO_4$ (pH 3.5) from 6–18 min postinjection and continuing isocratically with 1 M $NH_4H_2PO_4$ to 40 min. An on-line radioactive flow detector (Flo-one Beta, Radiomatic Instruments, Tampa, FL) was used to continuously monitor the radioactivity of the effluent. Eluted inositol phosphates were identified by comparison of their retention times to those of [^{3}H]Ins phosphate standards (New England Nuclear). Inositol 1,4,5-trisphosphate (InsP_3) levels were also measured by a D-*myo*-[^{3}H]inositol 1,4,5-trisphosphate assay system purchased from Amersham. This highly sensitive assay is based on the competition between unlabeled InsP_3 in the cellular extracts and a fixed quantity of a high specific activity [^{3}H]InsP_3 tracer for a limited number of binding sites on an InsP_3-specific binding protein (22, 23).

Analysis of Activation of Tyrosine Protein Kinase by Immunoblotting. Tyrosine protein kinase activation in human B-cell precursors was measured by immunoblotting with a highly specific rabbit anti-phosphotyrosine antibody, as described (24, 25). Controls included phosphate-buffered saline- or rhG-CSF-treated negative control samples as well as control samples stimulated with phorbol 12-myristate 13-acetate (PMA; 100 ng/ml; Sigma) plus the calcium ionophore ionomycin (100 nM; Calbiochem). In some experiments, cells were preincubated for 1 hr at 37°C with 30 or 100 μg of genistein per ml (ICN) or 100 μM sodium orthovanadate (Sigma) followed by rhIL-7 stimulation in the continued presence of genistein or vanadate.

RESULTS AND DISCUSSION

rhIL-7 Supports Clonal Proliferation of Human BCPs. We observed differences in IL-7 responsiveness among B-lineage LPC populations at discrete developmental stages, suggesting that functional IL-7Rs may be acquired in an orderly fashion and expressed only during the very early phases of human B-cell ontogeny. As shown in Table 1, rhIL-7 stimulated *in vitro* colony formation by immature LPCs with germ-line immunoglobulin heavy-chain genes, including multiphenotypic LPCs from 2 of 2 pro-B/T/M acute leukemia (AL) patients, biphenotypic LPCs from a fetal liver pro-B/T-cell line and a pro-B/T acute lymphoblastic leukemia (ALL) patient, and B-lineage LPCs from 3 of 3 fetal liver pro-B-cell lines. Most of the IL-7-responsive LPCs showed stimulated colony formation in the presence of rhIL-3, but none responded to rhIL-5 or rhIL-6. The IL-7 responsiveness did not depend on the IL-3 responsiveness since FL112 pro-B cells unresponsive to rhIL-3 were able to proliferate in the presence of rhIL-7 (Table 1). In contrast to its growth stimulatory effects on immature LPC populations, rhIL-7 failed to stimulate more mature B-lineage LPCs with rearranged immunoglobulin heavy-chain genes from 18 of 23 BCP (pre-pre-B or pre-B) ALL patients and 4 of 4 BCP (pre-pre-B or pre-B) ALL cell lines, 4 of 5 fetal livers, and 2 of 3 fetal bone marrows. rhIL-7 further failed to stimulate colony formation by mature B cells from 12 non-Hodgkin lymphoma patients, 2 Burkitt lymphoma cell lines, and 4 tonsils.

Human Pro-B Cells Express High-Affinity Receptors for IL-7. Recently, cDNA clones encoding the human and murine IL-7R were isolated (26, 27). To confirm that the biological effects of rhIL-7 on human pro-B cells were mediated by specific membrane receptors for IL-7, we used radioiodinated rhIL-7 (Fig. 1 *Inset A*) as an IL-7R-specific probe and correlated IL-7R expression in ligand binding assays with IL-7 responsiveness in proliferation assays. ^{125}I-rhIL-7 exhibited specific binding to FL112 fetal liver pro-B cells that was saturable with increasing concentrations of the radioiodinated ligand (Fig. 1). By comparison, NALM-6 pre-B ALL cells showed no specific ^{125}I-rhIL-7 binding (Fig. 1). Scatchard plot analysis of the specific ^{125}I-rhIL-7 equilibrium binding data for FL112 pro-B cells yielded a straight linear regression line, indicating the existence of a single class of high-affinity IL-7Rs (Fig. 1 *Inset B*). The calculated affinity constant (K_a) was 1.6 × 10^9 M^{-1} with

Table 1. *In vitro* proliferative responses of human lymphoid precursor cells to rhIL-7

Cell type	Colonies per 10^5 cells: No growth factor	rhIL-7	rhIL-3
Multiphenotypic LPC			
AK, CD7$^+$CD10$^+$CD13$^+$CD19$^+$, IgH:GG, AL pre-B/T/M	534 (484, 584)	1372 (1168, 1576)	1406 (1352, 1460)
SH, CD7$^+$CD10$^+$CD13$^+$CD19$^+$, IgH:GG, AL pre-B/T/M	64 (56, 72)	153 (125, 180)	160 (159, 161)
Biphenotypic LPC			
FL8.2., CD2$^+$CD10$^+$CD19$^+$C$_\mu^-$sIg$^-$, IgH:GG, FL pro-B/T	475 (403, 547)*	642 (608, 676)*	557 (548, 565)*
ES, CD2$^+$CD10$^+$CD19$^+$C$_\mu^-$sIg$^-$, IgH:RD, ALL pre-B/T	0 (0, 0)	178 (171, 185)	37 (29, 45)
B-lineage LPC			
FL112, CD10$^+$CD19$^+$CD72$^+$C$_\mu^-$sIg$^-$, IgH:GG, FL pro-B	14 (12, 16)*	1330 (1256, 1404)*	10 (8, 12)*
FL113, CD10$^+$CD19$^+$CD72$^+$C$_\mu^-$sIg$^-$, IgH:GG, FL pro-B	48 (40, 56)*	322 (284, 360)*	270 (236, 304)*
FL114, CD10$^+$CD19$^+$CD72$^+$C$_\mu^-$sIg$^-$, IgH:GG, FL pro-B	196 (182, 210)*	275 (274, 276)*	ND
FBM123, CD10$^+$CD19$^+$C$_\mu^-$sIg$^-$, IgH:GR, FBM pre-pre-B	0 (0, 0)	19 (15, 22)	107 (98, 116)
MC, CD10$^+$CD19$^+$CD72$^+$C$_\mu^-$sIg$^-$, IgH:RR, ALL pre-pre-B	0 (0, 0)	40 (39, 41)	58 (42, 73)
BJ, CD10$^+$CD19$^+$CD72$^+$C$_\mu^-$sIg$^-$, IgH:RR, ALL pre-pre-B	0 (0, 0)	62 (53, 71)	7 (6, 7)
JM, CD10$^+$CD19$^+$CD72$^+$C$_\mu^-$sIg$^-$, IgH:RR, ALL pre-pre-B	0 (0, 0)	198 (116, 280)	678 (628, 728)
JB, CD10$^+$CD19$^+$CD72$^+$C$_\mu^-$sIg$^-$, IgH:RR, ALL pre-pre-B	42 (27, 56)	200 (184, 216)	200 (156, 244)

Results from IL-7-responsive cases are shown as the mean number of colonies per 10^5 or 10^4 cells plated in duplicate Petri dishes. Numbers in parentheses represent actual colony numbers in individual dishes. C_μ, constant region μ-chain heavy chain gene; ND, not determined.

*Results expressed as colonies per 10^4 cells.

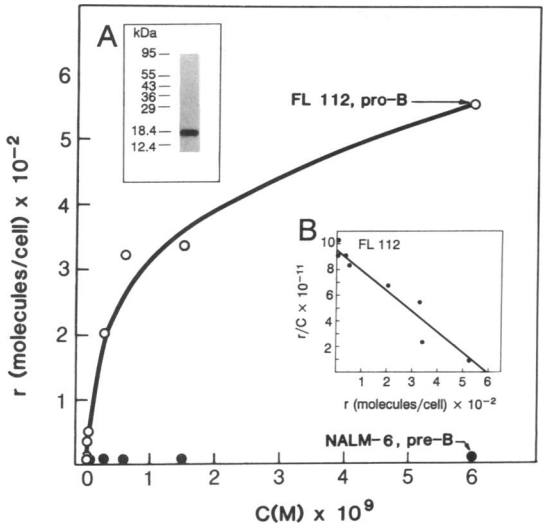

FIG. 1. Detection and characterization of IL-7Rs on human B-cell precursors. The radiochemical purity of ^{125}I-rhIL-7 was confirmed as described (15) by analytical SDS/PAGE and autoradiography that revealed a single band with an apparent mass of 17.5 kDa, which represented >99% of the radioactivity in the preparation (*Inset A*). The indicated amounts of ^{125}I-rhIL-7 were incubated with FL112 fetal liver pro-B cells or Nalm-6 ALL pre-B cells (50×10^6 cells per sample in duplicate) for 24 hr at 4°C in the presence and absence of 1000-fold unlabeled rhIL-7, and Scatchard plot analysis of the specific equilibrium binding data was performed to characterize IL-7Rs as described (15). Data points represent mean values obtained from three independent experiments. (*Inset B*) Specific equilibrium binding data for FL112 cells when transformed and replotted in the Scatchard coordinate system. Results are shown as the number of IL-7 receptors per cell. $K_a = 1.6 \times 10^9$ M^{-1}; $K_d = 6.2 \times 10^{-14}$ M; $r = -0.96$; $n = 596$. C, free ^{125}I-rhIL-7 concentration; r, number of ^{125}I-rhIL-7 molecules specifically bound per cell.

596 specific IL-7 binding sites per cell. The binding of ^{125}I-rhIL-7 to biphenotypic LPCs from FL8.2 cell line, pro-B cells from FL112 and FL113 cell lines, as well as pre-pre-B cells from 2 of 10 patients (V.L. and R.H.) was significantly blocked by a 1000-fold excess of unlabeled rhIL-7, but not by a 1000-fold excess of unlabeled rhG-CSF or rhIL-6 (data not shown). In contrast to the immature BCP populations, neither pre-B cells from 2 Cμ^+ ALL cell lines nor mature B cells from 2 Burkitt lymphoma cell lines (Raji, Namalwa) and one well-differentiated lymphocytic lymphoma patient showed significant ^{125}I-rhIL-7 binding, suggesting that functional IL-7Rs may be acquired in an orderly fashion and expressed only during the very early stages of human B-cell ontogeny. Cells from 12 of 12 patients that did not bind ^{125}I-rhIL-7 did not show any increased DNA synthesis, as measured by quantitative DNA flow cytometry or augmented colony formation in response to rhIL-7, whereas cells from 5 of 5 patients that displayed significant ^{125}I-rhIL-7 binding responded with either increased DNA synthesis only or with increased DNA synthesis as well as augmented colony formation (data not shown). Thus, ^{125}I-rhIL-7 binding correlated with IL-7 responsiveness. These findings indicate that functional IL-7Rs were detected in ligand binding assays and support the notion that the growth stimulatory effects of rhIL-7 are mediated via specific IL-7Rs.

rhIL-7 Induces Tyrosine Phosphorylation and Phospholipase C Activation in Human Pro-B and Pro-B/T LPC Populations. Many hormones, neurotransmitters, and growth factors such as epidermal growth factor, platelet-derived growth factor, and B-cell growth factor exert their biologic activity in part by stimulation of inositol phospholipid turnover (19, 28). To study the effects of ligand occupation of the IL-7R on inositol phospholipid turnover in IL-7-responsive human pro-B and pre-pre-B cells, we first measured the levels of inositol phosphates in FL112 pro-B cells by loading these cells with [^{3}H]Ins and separating the extracted [^{3}H]Ins phosphates by high-performance liquid chromatography (HPLC) on a strong anion-exchange column (Fig. 2). The incubation of FL112 cells with rhIL-7 (10 ng/ml) for 30 sec resulted in a 2.1-fold increase in InsP_3 and a 5.2-fold increase in inositol 1,3,4,5-tetrakisphosphate (InsP_4), as well as a 13% increase in inositol 1,4-bisphosphate (InsP_2) (see Fig. 4). To better quantitate and more accurately study the kinetics of rhIL-7-stimulated inositol phospholipid turnover and elevation of InsP_3 levels, we used a highly specific and quantitative [^{3}H]InsP_3 assay system (Amersham). rhIL-7 stimulated a rapid increase in the production of InsP_3 by IL-7-responsive FL112 pro-B cells (Fig. 3*A*), FL113 pro-B cells (Fig. 3*B*), FL-8.2 biphenotypic pro-B/T cells (Fig. 3*C*), and FL114 pro-B cells (Fig. 3*E*). The observed effects on InsP_3 levels in these immature LPCs appeared to be specific for rhIL-7, since we found no activity with rhIL-3, rhIL-4, rhIL-5, rhIL-6, or rhG-CSF. rhIL-7 did not induce inositol phospholipid turnover in pre-pre-B or pre-B cells that did not express IL-7Rs (Fig. 3*D*).

Tyrosine phosphorylation is thought to regulate the activity of phosphatidylinositol-specific phospholipase C (PI-PLC) and thus inositol phospholipid turnover (29–31). The activation of PI-PLC in fibroblasts by epidermal growth factor or platelet-derived growth factor as well as the activation of PI-PLC in T lymphocytes by engagement of the T-cell antigen receptor are preceded by phosphorylation of PI-PLC on tyrosine residues (29–31). Therefore, it was conceptually important to determine whether stimulation of human B-cell precursors by rhIL-7 could result in the activation of a tyrosine protein kinase. As shown in Fig. 3*E*, the tyrosine kinase inhibitor genistein (30 μg/ml) prevented the rhIL-7-stimulated production of InsP_3 in FL114 pro-B cells. These findings provided strong albeit circumstantial evidence that tyrosine phosphorylation is an important step in the generation of the IL-7R-linked mitogenic signal in human B-cell precursors. We next performed immunoblotting experiments with a polyclonal antiserum specific for phospho-

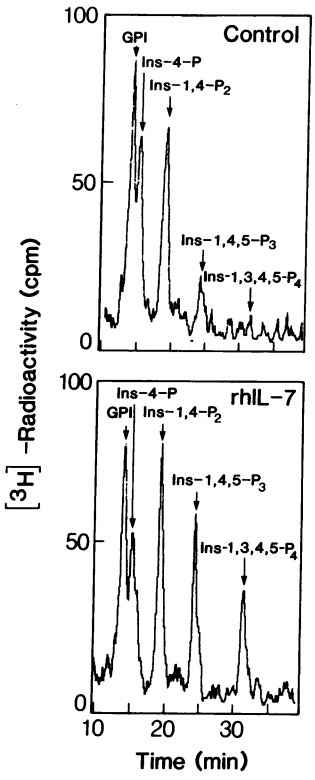

FIG. 2. Separation by HPLC of [^{3}H]Ins-labeled metabolites in rhIL-7-stimulated FL112 pro-B cells. Results are from one of two independent experiments that yielded similar results. (*Upper*) HPLC elution profiles of [^{3}H]Ins-labeled metabolites extracted from sham-treated FL112 cells. The retention times (amounts in cpm) were 4.3 min (287,447 cpm) for [^{3}H]Ins, 14.4 min (854 cpm) for glycerophosphatidylinositol (GPI), 15.4 min (709 cpm) for [^{3}H]Ins 4-phosphate, 19.4 min (784 cpm) for [^{3}H]InsP_2, 24.6 min (348 cpm) for [^{3}H]InsP_3, and 31.8 min (89 cpm) for [^{3}H]InsP_4. (*Lower*) HPLC elution profiles of [^{3}H]Ins-labeled metabolites extracted from rhIL-7-treated FL112 cells. The retention times (amounts in cpm) were 4.3 min (264,377 cpm) for [^{3}H]Ins, 14.2 min (841 cpm) for GPI, 15.3 min (761 cpm) for [^{3}H]Ins 4-phosphate, 19.5 min (885 cpm) for [^{3}H]InsP_2, 24.5 min (712 cpm) for [^{3}H]InsP_3, and 31.5 min (463 cpm) for [^{3}H]InsP_4.

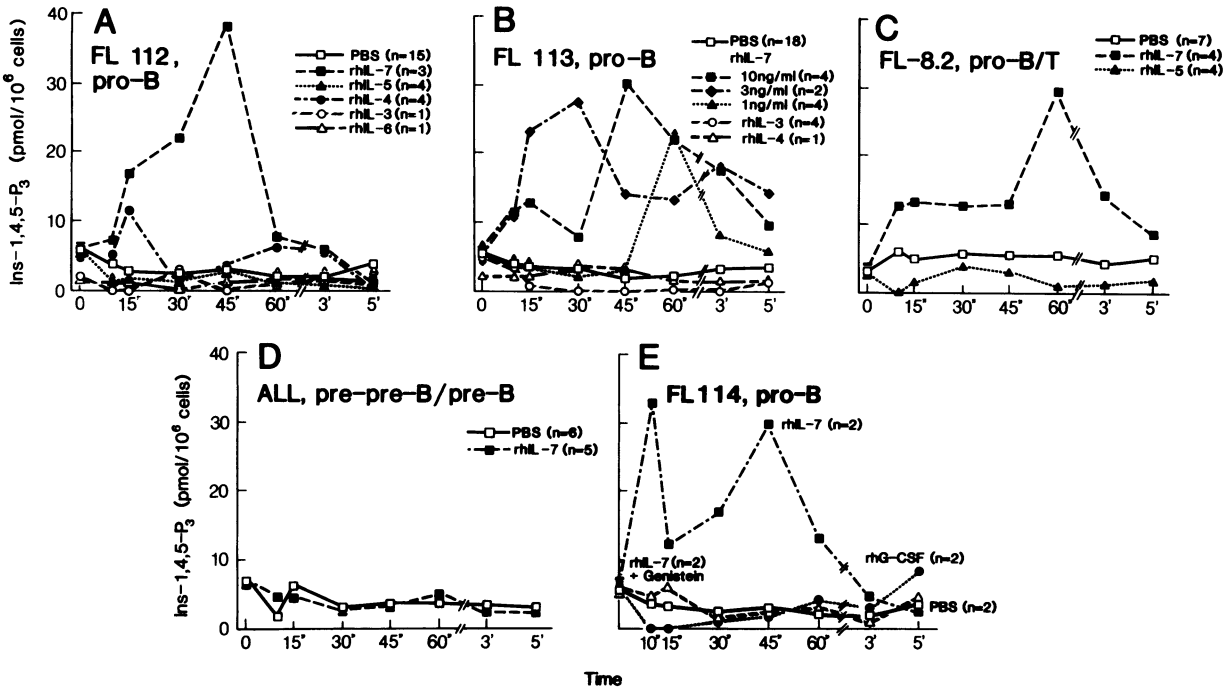

FIG. 3. (*A–D*) Magnitude and kinetics of rhIL-7-stimulated inositol phospholipid turnover in human B-cell precursors. Cells (5×10^6 cells per ml per tube) in MEM were either sham-treated with phosphate-buffered saline (PBS) or stimulated with rhIL-3, rhIL-4, rhIL-5, rhIL-6, or rhIL-7 at the indicated concentrations. Subsequently, the InsP_3 levels were determined at the indicated time points. The InsP_3 responses of the LPC populations were analyzed in several independent experiments (indicated by the *n*), each performed in quadruplicate. Results are expressed as the mean pmol amounts of InsP_3 per 10^6 cells. The calculated standard errors did not exceed 5% of the mean values. (*E*) Effects of the tyrosine kinase inhibitor, genistein, on rhIL-7-stimulated inositol phospholipid turnover in human B-cell precursors. FL114 pro-B cells (5×10^6 cells per ml per tube) in MEM were either sham-treated with PBS (negative baseline control) or stimulated with rhIL-7 (1 ng/ml), or rhIL-7 (1 ng/ml) plus genistein (30 μg/ml). Subsequently, the InsP_3 levels were determined at the indicated time points. rhG-CSF (10 ng/ml) served as a further negative control. *n*, Number of independent experiments performed. Results are expressed as the mean pmol amounts of InsP_3 per 10^6 cells. The calculated standard errors did not exceed 5% of the mean values.

tyrosine. As evidenced in Fig. 4*A*, stimulation of FL112 cells with rhIL-7 resulted in a transient increase in tyrosine phosphorylation of multiple electrophoretically distinct phosphoproteins. In particular, the 69-, 76-, 94-, and 150-kDa substrates were strongly phosphorylated. rhIL-7 further induced low levels of tyrosine phosphorylation on the 35-, 53-, 55-, 62-, 170-, and 190-kDa substrates. The induction of tyrosine phosphorylation in response to rhIL-7 could be

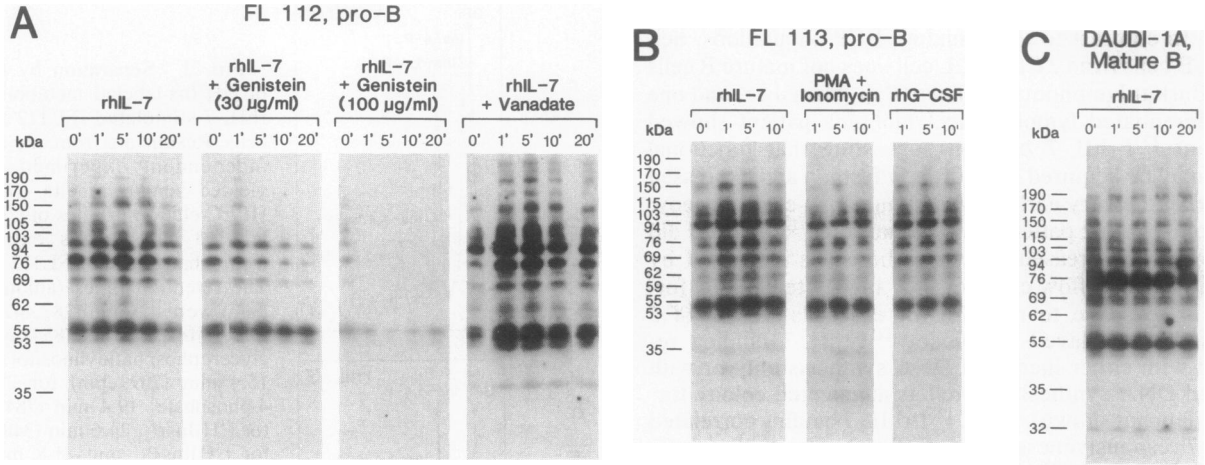

FIG. 4. rhIL-7 induces tyrosine phosphorylation of multiple electrophoretically distinct cellular substrates in human B-cell precursors. (*A*) FL112 pro-B cells were stimulated with rhIL-7 (1 ng/ml) in the presence or absence of the tyrosine kinase inhibitor genistein (30 or 100 μg/ml) or the tyrosine phosphatase inhibitor sodium orthovanadate (100 μM). Subsequently, cells were lysed with hot SDS lysis buffer, and equivalent amounts of protein were loaded on a 10.5% polyacrylamide gel, electrophoresed overnight, transferred to immobilon membrane, incubated with anti-phosphotyrosine antibody (0.5 μg/ml) and ^{125}I-labeled protein A before exposure to x-ray film as described (24, 25). The reactivity of anti-phosphotyrosine antibody with whole cell lysates of FL112 cells was completely blocked by the hapten phenylphosphate (40 mM) but not by 40 mM phosphoserine or 40 mM phosphothreonine (data not shown). (*B*) FL113 pro-B cells were treated with rhIL-7 (1 ng/ml), PMA (100 ng/ml) plus ionomycin (100 nM), or rhG-CSF (10 ng/ml) (negative control) and subsequently assayed for tyrosine phosphorylation as in *A*. (*C*) Surface IL-7R$^-$ Nalm-6 pre-B cells and Daudi-1 mature B cells, which do not show a proliferative response to rhIL-7, were studied for rhIL-7-induced tyrosine phosphorylation in an identical manner as FL112 and FL113 pro-B cells. Molecular masses (in kDa) of the phosphotyrosyl protein substrates were calculated from prestained molecular size markers run as standards.

abrogated by genistein (Fig. 4*A*), but it was not affected by the protein synthesis inihibitor cycloheximide or the serine/threonine kinase inhibitor H7, which inhibits protein kinase C, cAMP-dependent protein kinase, as well as cGMP-dependent protein kinase (data not shown). Tyrosine phosphorylation of the cellular substrates in FL112 cells peaked at 5 min and then rapidly declined to basal levels. The rapid dephosphorylation of the phosphotyrosyl proteins following rhIL-7-induced transient phosphorylation indicated that active tyrosine protein phosphatases may be involved in modulation of the IL-7R-linked signals in human B-cell precursors. In support of this hypothesis, the potent tyrosine protein phosphatase inhibitor sodium vanadate (100 μM) markedly enhanced protein tyrosine phosphorylation after rhIL-7 stimulation, probably by preventing the rapid dephosphorylation reaction (Fig. 4*A*).

Enhanced tyrosine phosphorylation after rhIL-7 stimulation was also observed in FL113 pro-B cells with a pattern very similar to that found in FL112 pro-B cells. As shown in Fig. 4*B*, the 76-, 94-, and 150-kDa substrates were strongly phosphorylated and low levels of tyrosine phosphorylation were induced on the 59-, 62-, 69-, 103-, 115-, 170-, and 190-kDa substrates. These effects were specific for rhIL-7 since (*i*) the phorbol ester PMA plus the calcium ionophore ionomycin or rhG-CSF did not induce tyrosine phosphorylation in IL-7R$^+$ pro-B cells, and (*ii*) rhIL-7 did not induce enhanced tyrosine phosphorylation in IL-7R$^-$ mature B cells (Fig. 4*C*).

In summary, this study examined the expression and function of IL-7Rs during early stages of human lymphocyte development as well as the mechanism of transmembrane signaling used by the IL-7R. Our data show that rhIL-7 directly stimulates proliferation of clonal populations of human multiphenotypic and biphenotypic LPCs as well as pro-B and some pre-pre-B cells but not pre-B cells or B cells. These findings prompt the hypothesis that human BCPs acquire functional IL-7Rs in a programmed order at the pro-B stage preceding the rearrangement of immunoglobulin heavy-chain genes, and they lose IL-7Rs prior to the expression of immunoglobulin genes at the pre-B stage. We provide evidence that the mitogenic transmembrane signal triggered by rhIL-7 is intimately linked to an active tyrosine protein kinase pathway and stimulates inositol phospholipid turnover producing $InsP_3$ as a second messenger. Since the IL-7R does not contain a tyrosine protein kinase domain (26), it may be noncovalently associated with a tyrosine protein kinase similar to the IL-2R (32). These experiments extend previous studies on the regulatory roles of hematopoietic growth factors in human B-lineage lymphopoiesis.

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