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Interleukin 7 receptor engagement stimulates tyrosine phosphorylation, inositol phospholipid turnover, proliferation, and selective differentiation to the CD4 lineage by human fetal thymocytes

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ABSTRACT **The purposes of this study were to elucidate the effects of recombinant human interleukin 7 (rhIL-7) on proliferation as well as differentiation of human fetal thymocytes and to analyze the biochemical nature of the IL-7 receptor-linked transmembrane signal. In the absence of costimulants, rhIL-7 stimulated the *in vitro* proliferation and colony formation of $CD4^+CD8^+$ double-positive immature fetal thymocytes. Furthermore, rhIL-7 promoted partial differentiation of immature thymocytes with a selective advantage for the development of $CD4^+CD8^-$ single-positive thymocytes. Our observations suggest that IL-7 likely has an important regulatory role during the earliest stages of human T-cell ontogeny. Stimulation of fetal thymocytes with rhIL-7 resulted in enhanced tyrosine phosphorylation of three distinct phosphoproteins with molecular masses of 72, 98, 123, and 190 kDa and induced a rapid and biphasic increase in the production of inositol 1,4,5-trisphosphate, which was inhibitable by the tyrosine protein kinase inhibitor genistein. Thus, the transmembrane signal triggered by engagement of the IL-7 receptor is intimately linked to a functional tyrosine protein kinase pathway and stimulates the inositol phospholipid turnover and proliferation, as well as selective differentiation to the CD4 lineage, by human fetal thymocytes.**

Human T lymphocytes are regulated at multiple levels by a network of hematopoietic cells and cytokines. Although the regulatory events during the late stages of human T-cell ontogeny by CD3/Ti and interleukin (IL)-2 have become better understood, the signals governing the proliferation or maturation of prothymocytes and thymocytes during the early developmental stages in T-lineage lymphopoiesis remain unclear (1–3).

IL-7 is a stromal cell-derived pleiotropic cytokine with lymphoid precursor cell growth-promoting activity (4–9). Recently, a cDNA clone encoding biologically active human IL-7 protein was isolated by cross-species hybridization with the homologous murine clone (10). A comparison of the human and murine IL-7 amino acid sequences has revealed 60% homology (10), and recombinant human (rh) IL-7 (rhIL-7) has been shown to have a marked T-cell stimulatory activity (11). There is circumstantial evidence that IL-7 may play an important role in the regulation of human T-lymphocyte development (10–12). This evidence includes mitogenic and comitogenic effects of rhIL-7 on human T lymphocytes (11) as well as the expression of IL-7 message in human thymus (10). However, very little is known about the involvement of IL-7 in intrathymic education of human thymocytes or its effects on proliferation and differentiation of T-cell precursor populations corresponding to sequential stages of early human T-cell ontogeny. In this report, we examine the biologic effects of rhIL-7 on fetal thymocytes as well as the biochemical nature of the rhIL-7-mediated transmembrane signal.

MATERIALS AND METHODS

Human Fetal Thymocytes. Human fetal thymuses ($n = 9$) from prostaglandin-induced human abortuses of 18–22 weeks (mean ± SE = 21 ± 0.5 weeks) of gestational age were used following the guidelines of the University of Minnesota Committee on the Use of Human Subjects in Research for secondary use of pathological or surgical tissue.

Recombinant Cytokines. 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 (10). Purified rhIL-3 (4×10^6 units/mg) and rhIL-6 (4×10^6 units/mg) were provided by S. Clark (Genetics Institute, Cambridge, MA), and rhIL-2 (5×10^6 units/mg) was obtained from Cetus. Recombinant human granulocyte colony-stimulating factor (rhG-CSF) (1×10^8 units/mg) was provided by L. Souza (Amgen Biologicals).

Immunological Marker Analyses. The surface antigen profiles of fetal thymocytes were analyzed by direct immunofluorescence and multiparameter flow cytometry on a FACStar^{Plus} (Becton Dickinson Immunocytometry Systems, San Jose, CA) (13, 14), using various combinations of the phycoerythrin, fluorescein isothiocyanate, or allophycocyanin conjugates of the following monoclonal antibodies: anti-Leu-6/CD1, 9.6/CD2, G19.4/CD3, G17.2/CD4, 10.2/CD5, G3.7/CD7, G10.1/CD8, anti-$\alpha\beta$ T-cell antigen receptor (TCR)-1 and anti-$\gamma\delta$TCR-1. Anti-TCR antibodies were purchased from Becton Dickinson.

Colony Assays. Fetal thymocytes were assayed for colony formation in the presence of the indicated concentrations of recombinant cytokines by using a colony assay system previously described (15, 16).

Measurement of Inositol 1,4,5-Trisphosphate ($InsP_3$) Levels. Following rhIL-7 stimulation, $InsP_3$ levels were measured by using a D-*myo*-[^{3}H]inositol 1,4,5-trisphosphate assay system purchased from Amersham, as reported (17). In some experiments, cells were preincubated for 1 hr at 37°C with genistein (ICN) at 30 μg/ml (123 μM) or H7 at 110 μg/ml (300 μM) and

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Abbreviations: IL, interleukin; rhIL, recombinant human IL; TCR, T-cell antigen receptor; $InsP_3$, inositol 1,4,5-trisphosphate; MHC, major histocompatibility complex; PLC, phospholipase C, PI-PLC, phosphatidylinositol-specific PLC; rhG-CSF, recombinant human granulocyte colony-stimulating factor; IL-7R, IL-7 receptor.

then stimulated with rhIL-7 in the continued presence of genistein or H7.

Analysis of Activation of Protein-Tyrosine Kinase by Immunoblotting. Protein-tyrosine kinase activation in fetal thymocytes was measured by immunoblotting with a purified polyclonal rabbit anti-phosphotyrosine antibody, as described (17, 18). Controls included samples treated with (*i*) rhG-CSF (10 ng/ml), (*ii*) rhIL-2 (10 ng/ml), (*iii*) rhIL-6 (10 ng/ml), (*iv*) rhIL-7 (10 ng/ml) plus the tyrosine kinase inhibitor genistein (30 μg/ml) (19), (*v*) rhIL-7 (10 ng/ml) plus the broad-spectrum kinase inhibitor staurosporine (9.3 ng/ml = 20 nM), (*vi*) rhIL-7 (10 ng/ml) plus the serine/threonine-specific protein kinase C inhibitor H7 (110 μg/ml), or (*vii*) rhIL-7 (10 ng/ml) plus the protein synthesis inhibitor cycloheximide (100 μg/ml = 355 μM). Staurosporine and cycloheximide were purchased from Calbiochem. Genistein was purchased from ICN, and H7 was purchased from GIBCO/BRL.

RESULTS

Surface Antigen Profiles of Fetal Thymocytes. As illustrated in Table 1, a significant fraction of cells in the analyzed fetal thymuses (n = 9) were $CD1^+CD2^+CD4^+CD7^+CD8^+CD3^-$-$\alpha\beta TCR^-\gamma\delta TCR^-$ double-positive immature thymocytes. Very few cells were $CD4^+CD8^-\alpha\beta TCR^+$ or $CD4^-CD8^+$-$\alpha\beta TCR^+$ single-positive mature thymocytes, $CD4^-CD8^-$ double-negative immature thymocytes, or $CD7^+CD2^-$ prothymocytes.

Effects of rhIL-7 on *in Vitro* Proliferation of Fetal Thymocytes. The mitogenic effects of rhIL-7 on fetal thymocytes were evaluated in colony assays in the absence of costimulants. Spontaneous colony formation in the absence of rhIL-7 was observed in eight of nine fetal thymuses, with a mean plating efficiency of 310 ± 102 colonies per 10^5 cells. rhIL-7 stimulated fetal thymocyte colony formation in all nine cases, with a mean plating efficiency of 943 ± 212 colonies per 10^5 cells and a mean colony stimulation index of 4.9 ± 1.6 (Table 2). *In vitro* proliferation of fetal thymocytes could also be stimulated by rhIL-2 or rhIL-6, but these recombinant cytokines were usually not as mitogenic as rhIL-7 (Table 2).

Table 1. Surface antigen profiles of freshly isolated human fetal thymocytes

Immunophenotype	% cells, mean ± SE (n = 9)
$CD1^+$	90 ± 2
$CD2^+$	90 ± 3
$CD3^+$	27 ± 4
$\alpha\beta TCR^+$	14 ± 2
$\gamma\delta TCR^+$	0.4 ± 0.1
$CD4^+$	89 ± 4
$CD7^+$	75 ± 8
$CD8^+$	85 ± 3
$CD7^+CD2^-$	2 ± 1
$CD4^-CD8^-$	4 ± 3
$CD2^+CD7^+$	91 ± 2
$CD1^+CD2^+$	91 ± 2
$CD4^+CD8^+$	79 ± 9
$CD3^+\alpha\beta TCR^-$	17 ± 3
$CD1^+CD3^-$	65 ± 7
$CD1^-CD3^+$	2 ± 1
$CD3^+\alpha\beta TCR^+$	10 ± 2
$CD3^+\gamma\delta TCR^+$	0.4 ± 0.1
$CD4^+CD8^-$	9 ± 3
$CD4^-CD8^+$	8 ± 4

The surface antigen profiles of freshly isolated human fetal thymocytes were determined by direct immunofluorescence and multiparameter flow cytometry. Results are expressed as the mean (± SE) percentage of thymocytes with the indicated immunophenotypic feature(s).

Table 2. Effects of rhIL-7 on *in vitro* proliferation and differentiation of human fetal thymocytes

Fetal thymus no.	Mean no. of colonies per 10^5 cells (stimulation index)			
	No GF	rhIL-7	rhIL-2	rhIL-6
1	849	1296 (1.5)	1020 (1.2)	NT
2	0	74 (ND)	NT	NT
3	424	790 (1.9)	660 (1.6)	632 (1.5)
4	164	538 (3.3)	400 (2.4)	344 (2.1)
5	722	1786 (2.5)	NT	NT
6	327	1223 (3.7)	653 (2.0)	243 (0.7)
7	66	972 (14.7)	688 (10.4)	58 (0.9)
8	20	78 (3.9)	92 (4.6)	NT
9	220	1734 (7.9)	246 (1.1)	NT
Mean ± SE	310 ± 102	943 ± 212 (4.9 ± 1.6)	537 ± 118.1 (3.3 ± 1.3)	319 ± 120 (1.3 ± 0.3)

Cells were cultured in the presence of purified rhIL-7 (10 ng/ml) for 7 days and assayed for colony formation, as described (15, 16). Colony cells were routinely immunophenotyped, as described (15, 16), to confirm their lineage affiliation and to formally rule out the possibility of outgrowth of contaminating accessory cells of the colony cells in rhIL-7-stimulated cultures. Of the colony cells in rhIL-7-stimulated fetal thymocyte cultures, 90% ± 2% were $CD2^+CD7^+$, 50% ± 7% were $CD1^+CD7^+$, 56% ± 8% were $CD4^+CD8^+$, 35% ± 5% were $CD4^+CD8^-$, and 4% ± 2% were $CD4^-CD8^+$. Controls included unstimulated cultures as well as cultures stimulated with rhIL-2 (10 ng/ml) or rhIL-6 (10 ng/ml). Results are shown as the mean number of colonies per 10^5 fetal thymocytes plated in duplicate Petri dishes. Numbers in parentheses represent the colony stimulation indices. NT, not tested; ND, not determined; GF, growth factors.

These findings indicate that IL-7 may have a growth regulatory role in early stages of human T-cell ontogeny.

Virtually all colony cells (mean ± SE = 90% ± 2%) in rhIL-7-stimulated fetal thymocyte cultures were $CD2^+CD7^+$,

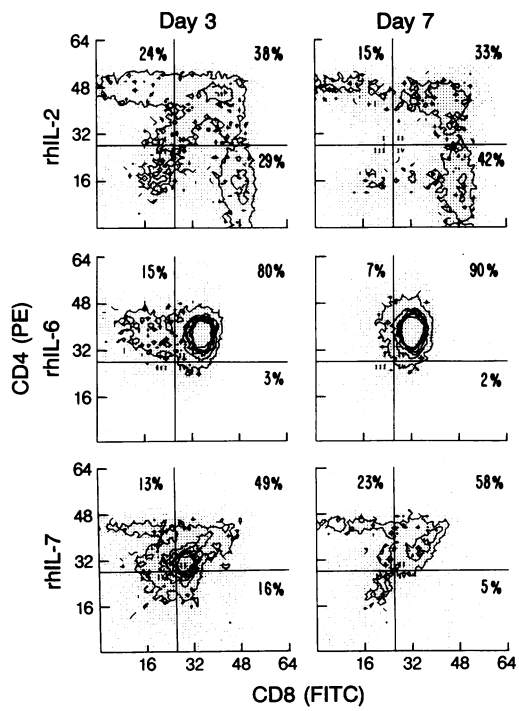

FIG. 1. Sequential multicolor flow cytometric analyses of fetal thymocytes stimulated with recombinant cytokines. Fluorescence-activated cell sorting-correlated two-parameter displays of ungated thymocytes from fetal thymus no. 4 two-color stained for CD8 and CD4 expression. PE, phycoerythrin; FITC, fluorescein isothiocyanate.

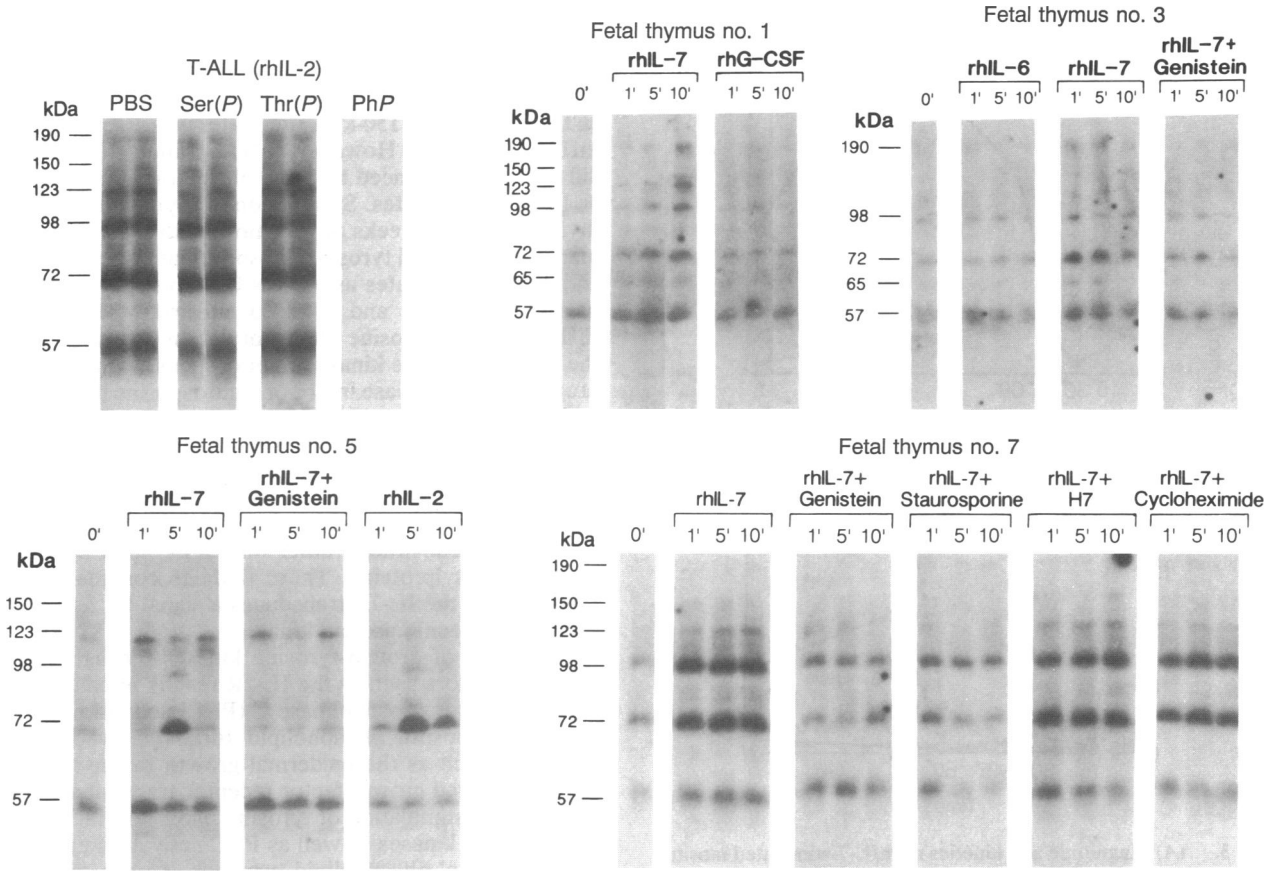

FIG. 2. rhIL-7 induces tyrosine phosphorylation of distinct cellular substrates in human fetal thymocytes. Fetal thymocytes were stimulated with rhIL-7 (10 ng/ml); with the control cytokines rhG-CSF (10 ng/ml), rhIL-2 (10 ng/ml), and rhIL-6 (10 ng/ml); or with rhIL-7 (10 ng/ml) plus genistein (123 μM), rhIL-7 (10 ng/ml) plus staurosporine (20 nM), rhIL-7 (10 ng/ml) plus H7 (30 μM), or rhIL-7 (10 ng/ml) plus cycloheximide (355 μM) for the times indicated and lysed with hot SDS lysis buffer. Equivalent amounts of protein were loaded on a 10.5% polyacrylamide gel, electrophoresed overnight, transferred onto a Immobilon poly(vinylidene difluoride) membrane, and incubated with anti-phosphotyrosine antibody (0.5 μg/ml) and ^{125}I-labeled protein A before exposure to x-ray film. To illustrate the specificity of the anti-phosphotyrosine antibody, whole cell lysates of rhIL-2 (10 ng/ml; 10 min)-stimulated T-lineage ALL blasts were prepared, run on a 10.5% polyacrylamide gel overnight, transferred onto Immobilon poly(vinylidene difluoride) membranes, and incubated with anti-phosphotyrosine antibody (0.5 μg/ml) in the presence or absence of 40 mM phosphoserine [Ser(*P*)], 40 mM phosphothreonine [Thr(*P*)], or 40 mM phenyl phosphate (Ph*P*). Subsequently, blots were developed with ^{125}I-labeled protein A and autoradiography. Molecular masses (in kDa) of the phosphotyrosylproteins were calculated from prestained molecular size markers. PBS, phosphate-buffered saline.

consistent with their T-lineage affiliation (legend of Table 2). Approximately one-half of these cells were $CD1^+CD7^+$ $CD4^+CD8^+$ double-positive immature thymocytes, and one-third were $CD4^+CD8^-$ single-positive mature thymocytes. Only 4% ± 2% $CD4^-CD8^+$ single-positive thymocytes were detected in rhIL-7-stimulated fetal thymocyte colonies (Table 2). Thus, rhIL-7 appeared to stimulate proliferation and partial differentiation of immature thymocytes with a selective advantage for the development of $CD4^+CD8^-$ single-positive thymocytes. We next performed sequential multicolor flow cytometric analyses of surface antigen profiles of fetal thymocytes stimulated in liquid cultures with rhIL-2, rhIL-6, or rhIL-7. rhIL-2, rhIL-6, and rhIL-7 were capable of stimulating the *in vitro* proliferation of fetal thymocytes, as evidenced by the increased cellularity of cytokine-stimulated liquid cultures (data not shown), and their stimulatory activity did not differ markedly. However, pronounced differences were noted in the surface antigen profiles of thymocytes in cultures stimulated with these cytokines. On day 3 of the culture period, a significant decrease in the percentage of $CD4^+CD8^+$ double-positive (Fig. 1) or $CD1^+CD3^-$ (data not shown) immature thymocytes was noted in rhIL-2-stimulated cultures. These immunophenotypic changes progressed until day 7 to yield a mixed population of $CD4^+CD8^+$ double-positive or $CD4^-CD8^-$ double-negative immature thymocytes and $CD4^+CD8^-$ or $CD4^-CD8^+$ single-positive mature thymocytes (Fig. 1). The percentage of $CD4^-CD8^+$ thymocytes in rhIL-2 stimulated cultures appeared to be 1.4- to 2.3-fold higher than the percentage of $CD4^+CD8^-$ thymocytes, which might reflect a selective growth advantage for the $CD4^-CD8^+$ thymocyte subpopulation. By comparison, rhIL-6 stimulated the *in vitro* proliferation of $CD4^+CD8^+$ double-positive immature thymocytes (Fig. 1). At the end of the 7-day culture period, the majority of cells in rhIL-6-stimulated cultures were double-positive immature thymocytes, and <10% were $CD4^+CD8^-$ or $CD4^-CD8^+$. Similarly, the majority of cells in rhIL-7-stimulated cultures were double-positive immature thymocytes. However, there was clear evidence of advanced differentiation as reflected by significantly lower percentages of $CD4^+CD8^+$ double-positive immature thymocytes and a higher percentage of $CD4^+CD8^-$ single-positive mature thymocytes in rhIL-7-stimulated cultures as compared to rhIL-6-stimulated cultures (Fig. 1). Thus, rhIL-7 stimulates the proliferation of fetal thymocytes, and unlike rhIL-2 or rhIL-6, it provides a selective positive pressure for their differentiation into $CD4^+CD8^-$ single-positive thymocytes.

rhIL-7 Induces Tyrosine Phosphorylation and Stimulation of Inositol Phospholipid Turnover in Fetal Thymocytes. The engagement of the human IL-7 receptor (IL-7R) on thymo-

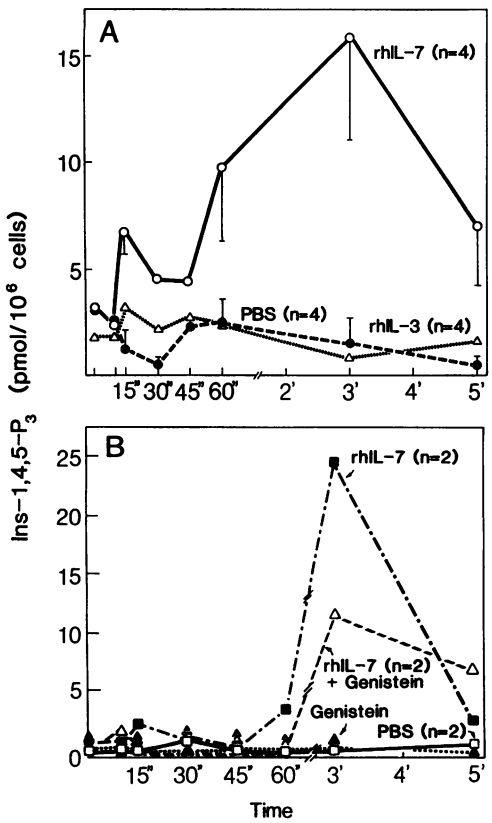

FIG. 3. (*A*) Magnitude and kinetics of rhIL-7-stimulated inositol phospholipid turnover in human fetal thymocytes. In four independent experiments, cells (5×10^6 cells per ml per tube) from fetal thymus nos. 1–4 in minimal essential medium (one thymus per experiment) were either sham-treated with phosphate-buffered saline (PBS) or stimulated with rhIL-3 (10 ng/ml) or rhIL-7 (10 ng/ml). Subsequently, the InsP_3 levels were determined at the indicated time points. Each experiment was performed in quadruplicate. Results are expressed as the mean amounts (pmol) of InsP_3 per 10^6 cells. The calculated standard errors are also indicated. (*B*) Effects of the tyrosine kinase inhibitor genistein on rhIL-7-stimulated inositol phospholipid turnover in human fetal thymocytes. In two independent experiments, cells (5×10^6 cells per ml per tube) from fetal thymus no. 7 and fetal thymus no. 9 in minimal essential medium were either sham-treated with phosphate-buffered saline (PBS) or stimulated with rhIL-7 (10 ng/ml), genistein (30 μg/ml), or rhIL-7 (10 ng/ml) plus genistein (30 μg/ml). Subsequently, the InsP_3 levels were determined at the indicated time points. Each experiment was performed in quadruplicate.

cytes from four representative fetal thymuses resulted in enhanced tyrosine phosphorylation of multiple substrates (Fig. 2). Stimulation of thymocytes from fetal thymus no. 1 (18 weeks of gestation) with rhIL-7 resulted in enhanced tyrosine phosphorylation of four electrophoretically distinct cellular substrates with molecular masses of 72, 98, 123, and 190 kDa. rhIL-7 further induced low-level tyrosine phosphorylation on a 57-kDa substrate. rhG-CSF, which was used as a negative control cytokine, did not induce tyrosine phosphorylation. In thymocytes from fetal thymus no. 3 (20 weeks of gestation), the engagement of the IL-7R with rhIL-7 resulted in enhanced tyrosine phosphorylation on the 72-, 98-, and 190-kDa substrates. In addition, low-level phosphorylation was induced on the 57-, 65-, 123-, and 150-kDa substrates. In contrast, no enhanced tyrosine phosphorylation was induced by stimulation of fetal thymocytes with rhIL-6 (Fig. 2). In thymocytes from fetal thymus no. 5 (21 weeks of gestation), stimulation with rhIL-7 resulted in markedly enhanced tyrosine phosphorylation on the 72-, 98-, and 123-kDa substrates and low-level phosphorylation on the 57- and 150-kDa substrates (Fig. 2). The induction of tyrosine phosphorylation in response to rhIL-7, especially on the 72-, 98-, and 150-kDa substrates was attenuated by the protein-tyrosine kinase inhibitor genistein. Tyrosine phosphorylation on the 72-, 98-, and 150-kDa substrates was also evident after rhIL-2 stimulation. However, rhIL-2-stimulated thymocytes did not show enhanced tyrosine phosphorylation on the 57- or 123-kDa substrates. Stimulation of thymocytes from fetal thymus no. 7 (21 weeks of gestation) with rhIL-7 resulted in markedly enhanced tyrosine phosphorylation on the 57-, 72-, and 98-kDa substrates as well as low-level phosphorylation on the 123-, 150-, and 190-kDa substrates (Fig. 2). This rhIL-7-induced tyrosine phosphorylation was attenuated by the protein-tyrosine kinase inhibitor genistein as well as the broad-spectrum kinase inhibitor staurosporine but not by the serine/threonine-specific protein kinase C inhibitor H7 (Fig. 2). Tyrosine phosphorylation was not affected by the protein synthesis inhibitor cycloheximide, which is consistent with the notion that the observed signal represents a tyrosine kinase-induced enhanced level of tyrosine phosphorylation on available substrates rather than a *de novo* synthesis of phosphotyrosylproteins. These findings conclusively demonstrate that the IL-7R transduces a signal for activation of a tyrosine-specific protein kinase.

Activation of protein-tyrosine kinases through some cytokine receptors such as the IL-2R results in cellular proliferation without phospholipase C (PLC) activation and stimulation of inositol phospholipid turnover, whereas other receptors such as the epidermal growth factor receptor or platelet-derived growth factor receptor transmit signals that induce both stimulation of tyrosine phosphorylation by protein-tyrosine kinases as well as PLC activation and stimulation of inositol phospholipid turnover. As shown in Fig. 3, rhIL-7 stimulated a rapid and biphasic increase in the production of InsP_3 by IL-7-responsive fetal thymocytes from four of four fetal thymuses. The observed effects on InsP_3 levels in fetal thymocytes were specific for rhIL-7, since we found no activity with rhIL-3.

Notably, the protein-tyrosine kinase inhibitor genistein (19) attenuated the rhIL-7-stimulated production of InsP_3 (Fig. 3*B*). By comparison, the serine/threonine-specific protein kinase C inhibitor H7 did not affect the rhIL-7-induced InsP_3 signal (data not shown). These findings provided strong, albeit circumstantial, evidence that tyrosine phosphorylation is an important step in the generation of the IL-7R-linked InsP_3 signal in fetal thymocytes, reminiscent of the tyrosine phosphorylation step during the TCR–CD3 complex-mediated activation of mature T cells (19).

DISCUSSION

The repertoire of TCR is shaped by positive and negative selection events occurring within the thymic microenvironment. The positive selection of antigen-specific class I or class II major histocompatibility complex (MHC)-restricted single-positive T cells in the thymus requires the specific low-affinity interaction of the $\alpha\beta$TCR with the restricting thymic MHC molecules. This interaction may be complemented by signals of accessory molecules on thymocytes or cytokines leading to further differentiation of double-positive $CD4^+CD8^+$ thymocytes with low-affinity TCR for self-MHC antigens into mature single-positive $CD4^+CD8^-$ and $CD4^-CD8^+$ T cells. Such complementary signals driving the positive selection and differentiation of thymocytes remain to be identified. It is possible that such signals also affect negative selection by either inactivating the apoptosis-inducing endonuclease (20, 21) and thus rescuing double-positive $CD4^+CD8^+$ thymocytes with high-affinity TCR for self-MHC antigens from programmed cell death or by activating the endonuclease and thus accelerating apoptosis. The

possible regulatory role of IL-7 in human T-cell ontogeny, which has been implicated by the abundant expression of IL-7 message in thymus as well as the broad spectrum T-cell precursor/T-cell stimulatory activity of IL-7, is as yet undefined. Here, we show that rhIL-7 not only stimulates the proliferation of prothymocytes and thymocytes, but it also provides a selective positive pressure for their differentiation into $CD4^+CD8^-$ single-positive thymocytes. Thus, the physiologic role of IL-7 in thymic differentiation may be to provide a complementary signal for proliferation and terminal maturation of thymocytes selected to differentiate into single-positive $CD4^+CD8^-$ T cells. These observed biologic effects of rhIL-7 are quite different from those of rhIL-2 or rhIL-6. rhIL-2 stimulates both proliferation and differentiation of thymocytes with partial advantage for the development of $CD4^-CD8^+$ thymocytes, whereas rhIL-6 is mitogenic to the vast majority of $CD4^+CD8^+$ immature thymocytes without inducing advanced differentiation.

Recent studies have provided strong evidence that tyrosine-specific protein kinases play a pivotal role in the control of early events leading to cell activation and proliferation upon ligation of a number of growth factor receptors. Tyrosine phosphorylation is thought to regulate the activity of phosphatidylinositol (PI)-specific PLC (PI-PLC) and thus inositol phospholipid turnover and calcium mobilization. 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 TCR are preceded by phosphorylation of PI-PLC on tyrosine residues (19, 22, 23). Importantly, intrathymic signaling in immature $CD4^+CD8^+$ murine thymocytes after engagement of TCR, CD4, or CD8 molecules results in activation of tyrosine kinases and leads to tyrosine phosphorylation of TCR ζ chain (19, 24–26). The signals for murine or human T-cell precursors mediated by IL-7 have not been biochemically identified. Our report provides evidence that the mitogenic transmembrane signal triggered by rhIL-7 in human fetal thymocytes is intimately linked to signal-transduction pathways that stimulate tyrosine phosphorylation of multiple substrates as well as inositol phospholipid turnover, producing $InsP_3$ as a second messenger. The role of tyrosine phosphorylation in rhIL-7-mediated stimulation of inositol phospholipid turnover was examined with a protein-tyrosine kinase inhibitor, genistein. Genistein prevented the rhIL-7-stimulated production of $InsP_3$, providing strong, albeit circumstantial, evidence that tyrosine phosphorylation is an important and possibly mandatory step in the generation of the IL-7R-linked transmembrane signal in fetal thymocytes, reminiscent of the tyrosine phosphorylation step during the TCR–CD3 complex-mediated activation of mature T cells (19).

Our findings indicate that IL-7 may play a pivotal regulatory role during the early stages of human T-cell ontogeny. While expanding the information from previous studies on the bioactivities of IL-7, these data should also promote future investigations of IL-7–IL-6, IL-7–IL-2, and IL-7–IL-4 interactions at the level of thymocyte populations corresponding to early stages of human T-lymphocyte development.

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