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In vitro effects of recombinant interleukin 7 on growth and differentiation of bone marrow pro-B- and pro-T-lymphocyte clones and fetal thymocyte clones

(lymphocyte development/lymphoid precursors/cell differentiation/interleukins/bone marrow stroma)

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ABSTRACT **We have studied the effects of recombinant (r) interleukin 7 (IL-7) on growth and differentiation of marrow pro-B-lymphocyte clones (CB/Bm7, LyD9, LyB9), marrow pro-T-lymphocyte clones (C4-77/3, C4-86/18, C4-95/16), and fetal thymocyte clones (FTH5, FTA2, FTD5) in the presence or absence of the bone marrow stroma clone RP.0.10, which was selected for its ability to promote differentiation of the pro-B clones. rIL-7 alone stimulated some DNA synthesis (measured by [^{3}H]thymidine uptake) but not actual growth (increase in cell number) of the pro-B clones. Antibodies against IL-4 and IL-6 or against receptors for IL-2, IL-3, and IL-5 did not inhibit this effect of rIL-7 on the pro-B clones. rIL-7 alone or in various combinations with other cytokines (from rIL-1α to rIL-6) could not induce differentiation of the pro-B clones into IgM$^+$ B cells regardless of the presence of lipopolysaccharide (LPS). The RP.0.10 marrow stroma cells by themselves do not support the growth of the pro-B clones. However, the pro-B clones grew when cultured with rIL-7 and monolayers of the RP.0.10 stroma cells. While the RP.0.10 stroma cells induced the pro-B clones to differentiate into IgM$^+$ B cells but not T3$^+$ T cells when cultured in the presence of LPS and rIL-3, the B-cell progenitor clones gave rise to significantly higher numbers of IgM$^+$ B cells (up to 63%) and to many more B cells expressing higher levels of surface IgM when cocultured with rIL-7, LPS, and RP.0.10 stroma cells. The pro-B clones also generated IgM$^+$ B cells (up to 20%) when cocultured with RP.0.10 stroma cells and rIL-7 in the absence of LPS. By using culture plates designed for testing requirements for cell–cell contact, we found that cell interactions between the pro-B cell and the marrow stroma cell are essential to induce rearrangement and expression of the immunoglobulin genes in the pro-B clones. Possible mechanisms to account for the remarkable effects of rIL-7 in the presence of RP.0.10 stroma cells on both growth and differentiation of the pro-B clones are discussed. Finally, rIL-7 alone or together with RP.0.10 stroma cells neither supported proliferation nor induced differentiation into T3$^+$ T cells or IgM$^+$ B cells of the marrow pro-T clones or the fetal thymocyte clones. In light of these findings, we postulate that the interaction of the pluripotential stem cell with marrow stroma cells like RP.0.10 and the availability of IL-7 could play a critical role in the commitment to develop along the B-lymphocyte pathway.**

Recently, a 25-kDa protein secreted by a simian virus 40-transformed bone marrow cell line was purified (1) and a cDNA encoding it was isolated (2). Both purified natural and recombinant (r) material were found to promote proliferation of bone marrow B-lymphocyte lineage cells (1, 2). As this molecule was found to be different from the known interleukins (from IL-1 to IL-6) it was called interleukin 7 (IL-7) (2). Other groups previously reported factors, secreted by bone marrow stroma cells acting on some pre-B cells, developed in long-term marrow cultures (3, 4). Whether they are related to IL-7 remains to be defined. To study further the biological activities of IL-7, we have examined the effects of rIL-7 on both growth and differentiation of continuously proliferating nontransformed clones representing the earliest stages of B- and T-lymphocyte development isolated from bone marrow of young mice and thymus of 14-day mouse embryos. Here we report and discuss the results of such experiments.

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MATERIALS AND METHODS

Antibodies. The following antibodies were used: fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgM (Southern Biotechnology Associates, Birmingham, AL), biotin-conjugated anti-B220 monoclonal antibody (mAb) [hybridoma-14.8 (5)], biotin-conjugated anti-mouse T3 ε chain [hybridoma-145-2C11 (6)], and FITC-labeled streptavidin (Amersham). Purified anti-mouse IL-4 mAb 11B1 (7) was purchased from Texstar Monoclonals (Dallas); rat anti-mouse IL-2 receptor [hybridomas 7D4 (8) and PC61 (9)], rat anti-mouse IL-5 receptor [hybridoma R52.120 (A. Rolink, F. Melchers, and R.P., unpublished data)], mouse anti-IL-3-sensitive mouse cells [hybridoma CC11 (10)], rat anti-IL-6 mAb [hybridoma 6B4 (11)], rat anti-mouse PgP-1 (hybridoma I42/5), and FITC-conjugated anti-rat IgG were from Southern Biotechnology Associates.

Cytokines. Purified human rIL-1α was a gift of Manfred Brockhaus (Hoffmann–La Roche, Basel), rIL-2, rIL-3, rIL-4, rIL-5, and rIL-6 were supernatants from X63Ag8 myeloma cells transfected with cDNAs encoding the respective interleukins (ref. 12) and the biological activity of each cytokine was tested in their respective assays as described (10, 13). One unit of activity was defined as the amount of supernatant given half of the maximal response. Mouse rIL-7 was supernatants from transfected HeLa cells with cDNA encoding IL-7 (2). The rIL-7 used in the present study contained 50,000 units of activity per ml as assessed in the assay described by Namen *et al.* (1).

Cell Lines. *Marrow pro-B clones.* The cellular, molecular, and functional properties of the CB/Bm7, LyB9, and LyD9 pro-B-lymphocyte clones were described (13, 14).

Marrow pro-T clones. The subclones C4-77/3, C4-86/18, and C4-45/16 derived from the marrow pro-T clones C4-77, C4-16, and C4-95, respectively (15), were used. They are able to repopulate thymuses of sublethally irradiated H-2-matched mice after intrathymic injection and to generate

Abbreviations: r, recombinant; IL, interleukin; FITC, fluorescein isothiocyanate; mAb, monoclonal antibody; LPS, lipopolysaccharide.

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both $LyT2^+$ $L3T4^-$ and $LyT2^-$ $L3T4^+$ $T3^+$ splenic lymphocytes after transfer into sublethally irradiated Scid mice (16).

Fetal thymocyte clones. The cellular and molecular characterization of the fetal thymocyte clones FTH5, FTD5, and FTA2 were described (17, 18).

Bone marrow stroma clones. The development and characterization of the RP.0.10 bone marrow stroma clone were described (19). RP.0.10 stroma cells were obtained from the marrow of an 8-week-old C57BL/6 mouse. Phenotypically, they are class I $MHC^{\pm}$ (MHC, major histocompatibility complex), class II MHC^-, Mac-1^-, Mac-2^-, Mac-3^+, BP-1^-, B-220^-, IgM^-. While the pro-B clones bind to RP.0.10 stroma cells, the stroma clone does not support cell division of the B-cell progenitor clones in the absence of exogenous growth factor. RP.0.10 stroma cells are able to promote differentiation of the pro-B clones into IgM^+ B lymphocytes (19), as well as of freshly isolated B-cell precursors from the marrow and fetal liver (R.P., unpublished results).

All marrow lymphoid progenitor clones were maintained in culture with IL-3-supplemented medium, the fetal thymocyte clones were propagated in IL-4-supplemented medium, and the RP.0.10 stroma clone was propagated in culture medium (Iscove's modified Dulbecco's medium plus 50 μM 2-mercaptoethanol), gentamycin (50 μg/ml), 2 mM L-glutamine, and fetal calf serum (5%; batch 537770, Ready Systems, Bad Zurzach, Switzerland) in the absence of exogenous growth factors. Culture medium prepared as indicated above was used in all the experiments.

Assay for Cell Proliferation. This was carried out as described (10, 13), where the response of 10^4 cells per microwell to several dilutions of rIL-7 (from 1:4 to 1:8192) was assessed by [^{3}H]thymidine uptake (1 μCi per well; 1 Ci = 37 GBq; TRA185MBq, Radiochemical Centre, Amersham) during the last 6 hr of a 2-day culture period.

As positive controls, the proliferative response of the marrow clones to rIL-3 (50 units/ml) and that of the fetal thymocyte clones to rIL-4 (50 units/ml) were also included in the assays.

The capacity of the mAbs against IL-4 (50 μg/ml), IL-2 receptor (mixture of PC61 and 7D4 mAbs at a final concentration of 10%), IL-5 receptor (50 μg/ml), IL-3 receptor (50 μg/ml), and IL-6 (final concentration, 20%) of inhibiting the proliferation of LyB9 cells driven by rIL-7 (final dilutions, 1:50, 1:100, and 1:1000) was tested by adding the antibodies at the beginning of the cultures established as indicated above. These antibodies used at the same concentrations as above inhibited by 70–95% the proliferative response driven by the respective interleukin using the following as target cells: FTH5 for IL-4, LyB9 for IL-3, B13 for IL-5, CTLL for IL-2, and the hybridoma B45 (kindly provided by A. Rolink, Basel Institute for Immunology) for IL-6.

Assays for Cell Differentiation. In one set of experiments, the capacity of rIL-7 alone or in combination with other interleukins of inducing differentiation of the lymphocyte progenitor clones into IgM^+ B cells and/or $T3^+$ T cells was studied as follows: the clones (10^5 cells per Costar well; three wells per group) were cultured in medium containing saturating concentrations of rIL-7 (final dilution, 1:100) alone or in combination with rIL-1α, rIL-2, rIL-3, rIL-4, rIL-5, and rIL-6 (final concentration, 100 units/ml) in the presence or absence of lipopolysaccharide (LPS) (40 μg/ml) in a final volume of 1 ml of culture medium per well at 37°C. At days 3 and 6 of culture, each well received 1 ml of freshly prepared medium supplemented with the respective interleukins. After 8–10 days of culture, the cells were harvested and washed, and the presence of cells expressing surface IgM, T3, or PgP-1 was determined by immunofluorescence staining and flow fluorocytometry (see below).

In another set of experiments, we tested the effects of rIL-7 on pro-B-cell differentiation in a clonal assay system recently developed by us (19). In this system, the pro-B clones are induced to differentiate *in vitro* into IgM^+ B lymphocytes when cocultured with the RP.0.10 marrow stroma clone, rIL-3, and LPS (19). The cultures were carried out in six-well Costar plates (cat. no. 3506) having monolayers of RP.0.10 stroma cells. Lymphocyte progenitor cells (10^5 cells) suspended in culture medium containing either rIL-7 (final dilution, 1:100) or rIL-3 (2–3 units/ml; higher concentrations of rIL-3 promote a high degree of growth but hinder the differentiation of the pro-B-cell clones) in a final volume of 2 ml were added to each Costar well (six wells per group). Two days later, LPS (40 μg/ml) was added to the cultures and they were incubated at 37°C for a total of 8–10 days. In initial experiments, we found that when the cultures that received rIL-7 were fed with 1 ml of medium containing rIL-7 after 4–5 days of initiation of the cultures, the number of viable cells recovered at the end of the cultures was significantly higher without impairing the differentiation of the pro-B cells into IgM^+ B lymphocytes. Therefore, we decided to perform all subsequent experiments following this protocol. The presence of IgM^+ B cells, B-220^+ cells, and $T3^+$ cells was assessed by immunofluorescence staining and flow cytometry (FACS).

In the third set of experiments, we made use of Costar plate wells equipped with membrane filters specially designed to test requirements for cell–cell interactions (Transwell, cat. no. 3408). In these experiments, monolayers of RP.0.10 stroma cells, LPS, and rIL-7 (at concentrations indicated above) were at the bottom of the wells and the LyB9 and CB/Bm7 pro-B-cell clones were placed on top of the filter membrane, which laid on the RP.0.10 stroma monolayer. Parallel cultures set up as detailed above where the pro-B cells can interact with the RP.0.10 stroma cells were also carried out for comparison and served as positive controls. After 8–10 days of culture, the lymphoid cells were collected and tested for the presence of IgM^+ B cells and $T3^+$ T cells by FACS analysis.

FACS Analysis. This was carried out as described (13, 15) with a FACScan instrument (Becton Dickinson). Direct or indirect fluorescence stainings were performed using FITC-labeled streptavidin (Amersham) or FITC-labeled goat anti-rat IgG as second-step reagent. Staining of cells with biotin-conjugated anti-T3 antibody was carried out in the presence of heat-inactivated normal hamster serum to avoid nonspecific binding. As positive controls, spleen cells from CBA/J mice were used. Negative controls were the cells stained with second-step reagent only. Dead cells were excluded from analysis by propidium iodide. Fluorescence emitted by single viable cells was determined using 4-decade logarithmic amplification and the data collected from 10^4 cells were analyzed with Consort 30 software.

RESULTS AND DISCUSSION

Effect of rIL-7 on pro-B Clones. First we tested rIL-7 on the growth of three pro-B clones (LyD9, CB/Bm7, LyB9). We found that rIL-7 alone stimulated little DNA synthesis (assessed by [^{3}H]thymidine uptake) on the pro-B clones, but it did not support actual growth (increase in cell numbers) of these cells. The response to rIL-7 alone by the pro-B clones was always <10% of those obtained with rIL-3 (Fig. 1). The DNA synthesis stimulated by rIL-7 was not inhibited by mAbs against IL-4 and IL-6 or against receptors for IL-2, IL-3, and IL-5 (Table 1). Parallel experiments showed that all the mAbs used at the same concentrations inhibited by 70–95% the action of the respective interleukin. Thus, rIL-7 stimulated DNA synthesis on the pro-B clones via IL-2, IL-3, IL-4, IL-5, and IL-6-independent pathways.

The capacity of rIL-7 alone or in combination with other recombinant interleukins (rIL-1α, rIL-2, rIL-3, rIL-4, rIL-5, and rIL-6) of inducing differentiation of the pro-B clones into

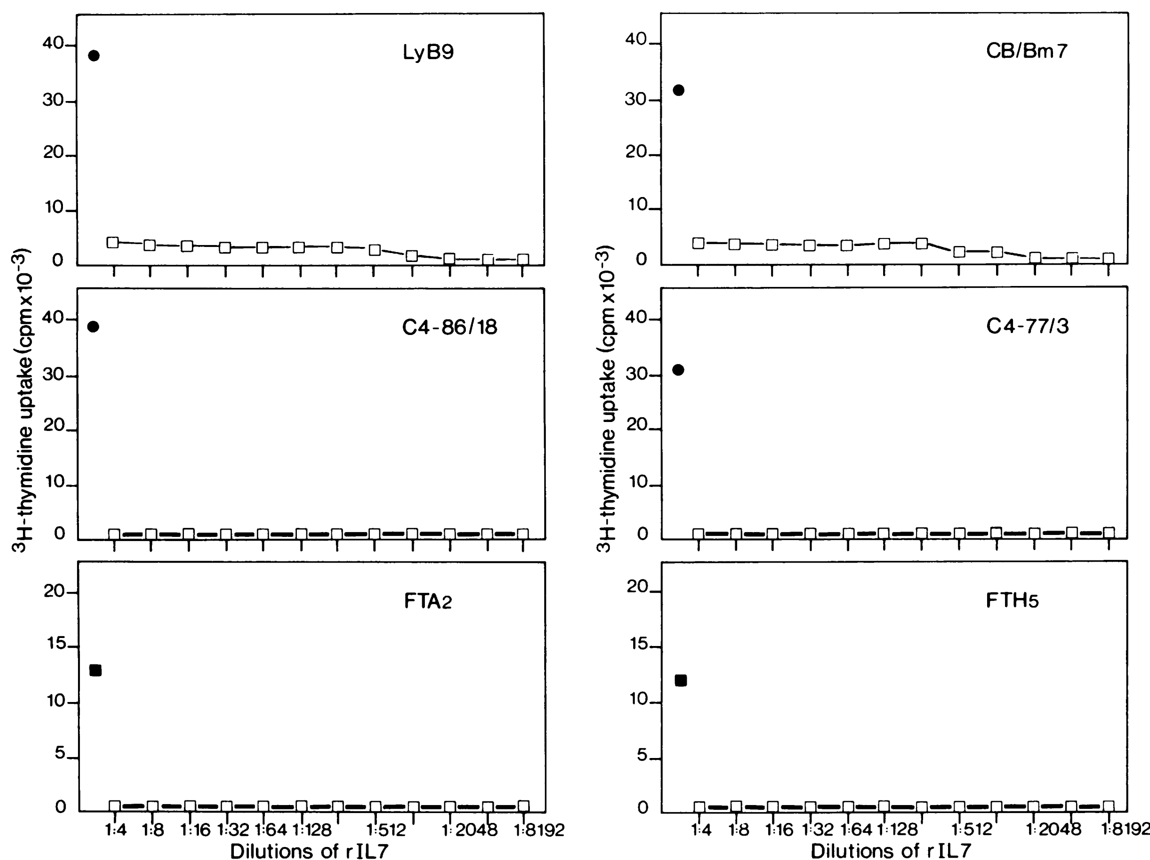

FIG. 1. Cell proliferation of the lymphoid precursor clones indicated in response to several dilutions of rIL-7 (□), rIL-3 (●) (50 units/ml), or rIL-4 (■) (50 units/ml) was assessed by [^{3}H]thymidine uptake during the last 6 hr of a 2-day culture period carried out at 37°C. Cells incubated in medium only gave FTH5 = 160 cpm, FTA2 = 199 cpm, LyB9 = 115 cpm, CB/Bm7 = 106 cpm, C4-77/3 = 94 cpm, and C4-86/18 = 297 cpm.

IgM$^+$ B cells or TCR/T3$^+$ T cells was studied as detailed in *Materials and Methods*. None of the several combinations tested could induce the pro-B clones to differentiate regardless of the presence of LPS (Table 2, group A).

Effects of rIL-7 in the Presence of the Bone Marrow Stroma Clone RP.0.10 on the pro-B Clones. Recently, we found that the pro-B clones selectively bind to some marrow stroma cells and took advantage of this property to isolate and establish in culture such marrow stroma cells. We also found that while the isolated marrow stroma clones promoted differentiation of the pro-B clones into IgM$^+$ B cells, they could not support growth of the B-cell progenitor in the absence of exogenous growth factors, unlike the findings obtained using heterogenous preparations of marrow-adherent stroma cells (19). In the experiments using the bone marrow stroma clones, such as RP.0.10, we found it necessary to include limiting amounts of rIL-3 (1–3 units/ml; higher concentrations promoted significant growth but hindered the differentiation of the pro-B clones) to allow the B-cell progenitor clones to survive and, hence, be able to respond to the differentiating stimuli of RP.0.10 marrow stroma cells (19).

Table 1. rIL-7 stimulates DNA synthesis on pro-B clones via IL-2-, IL-3-, IL-4-, IL-5-, and IL-6-independent pathways

mAb added to culture	[^{3}H]Thymidine uptake, cpm 1:50	1:100	1:1000
None	3238	2504	924
PC61 + 7C4 (anti-IL-2R)	3478	2220	806
CC11 (anti-IL-3R)	3666	2634	703
11B1 (anti-IL-4)	3196	2557	812
R52.120 (anti-IL-5R)	3104	2146	784
6B4 (anti-IL-6)	3373	2440	724

CB/Bm7 pro-B cells were cultured in the dilutions of rIL-7 indicated in the presence or absence of the mAbs PC61 + 7D4 (final concentration, 10%) CC11 (50 μg/ml), 11B1 (50 μg/ml), R52.120 (50 μg/ml), or 6B4 (final concentration, 20%) at 37°C for 2 days. R, receptor. Cell proliferation was determined by [^{3}H]thymidine uptake during the last 6 hr of culture. Parallel experiments showed that the mAbs at the concentrations tested inhibited by 75–95% the action of the respective interleukins.

We were interested in testing the effects of rIL-7 in this clonal system for pro-B-cell differentiation. Thus, parallel cultures consisting of monolayers of RP.0.10, pro-B cells, LPS, and either rIL-3 or rIL-7 were set up. The presence of IgM$^+$ B cells and/or T3$^+$ T cells was assessed by FACS analysis 8–10 days later. Two striking findings became apparent in these experiments. One was that the pro-B clones grew in the presence of both RP.0.10 stroma cells and rIL-7; the number of cells recovered after 3–4 days of culture increased from 6×10^5 input cells to 3.2–4.1×10^6 in six-well cultures. The number of viable cells recovered from cultures containing RP.0.10 stroma cells and rIL-3 were usually higher (4.8–7.3×10^6 cells in six-well cultures). The other remakable finding was that the pro-B clones when cocultured with RP.0.10 stroma cells, LPS, and rIL-7 gave rise to significantly higher numbers of IgM$^+$ B cells and to more B cells bearing higher levels of surface IgM than those generated by the same pro-B clones cocultured with RP.0.10 stroma cells, LPS, and rIL-3. The pro-B clones also generated IgM$^+$ B cells (up to 20%) when cultured with RP.0.10 stroma cells and rIL-7 in the absence of LPS. No T3$^+$ T cells were detected under either culture conditions in agreement with previous studies, showing that the pro-B clones cannot give rise to T lymphocytes either *in vivo* or *in vitro* (13, 14, 20). Fig. 2 illustrates these findings in the form of fluorescence histograms and

Table 2. Effects of rIL-7 on differentiation of lymphocyte progenitor clones

	Progenitor clone											
	LyB9		CB/Bm7		C4-77/3		C4-86/18		FTH5		FTA2	
	IgM$^+$	T3$^+$	IgM$^+$	T3$^+$	IgM$^+$	T3$^+$	IgM$^+$	T3$^+$	IgM$^+$	T3$^+$	IgM$^+$	T3$^+$
Group A												
rIL-7	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
rIL-7 + rIL-3	<1	<1	<1	<1	<1	<1	<1	<1	NP	NP	NP	NP
rIL-7 + rIL-1α	<1	<1	<1	<1	NP	NP	NP	NP	NP	NP	NP	NP
rIL-7 + rIL-4	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
rIL-7 + rIL-5	<1	<1	<1	<1	NP	NP	NP	NP	NP	NP	NP	NP
rIL-7 + rIL-6	<1	<1	<1	<1	NP	NP	NP	NP	NP	NP	NP	NP
rIL-7 + rIL-3 + rIL-1α	<1	<1	<1	<1	<1	<1	<1	<1	NP	NP	NP	NP
rIL-7 + rIL-3 + rIL-4	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
rIL-7 + rIL-3 + rIL-5	<1	<1	<1	<1	<1	<1	<1	<1	NP	NP	NP	NP
rIL-7 + rIL-3 + rIL-6	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
rIL-7 + rIL-3 + rIL-2	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
rIL-7 + rIL-3 + rIL-4 + rIL-5 + rIL-6	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
rIL-7 + rIL-4 + rIL-2 + rIL-6	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
rIL-7 + rIL-4 + rIL-5 + rIL-6 + rIL-1α	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Group B												
RP.0.10 cells + LPS + rIL-7	41–56	<1	48–63	<1	NP	NP	NP	NP	NP	NP	NP	NP
RP.0.10 cells + LPS + rIL-3	12–16	<1	13–19	<1	<1	<1	<1	<1	<1	<1	<1	<1
Group C												
RP.0.10 cells + LPS + rIL-7 (precluding cell interactions)	<1	<1	<1	<1								
RP.0.10 cells + LPS + rIL-7 (permissive for cell interactions)	37–43	<1	44–51	<1								

The capacity of the progenitor clones to differentiate into IgM$^+$ B cells or T3$^+$ T cells in response to the various stimuli indicated was studied as detailed in *Materials and Methods*. IgM$^+$ or T3$^+$ cells were determined by FACS analysis after 8–10 days of culture. In experiments of group A all cultures also received LPS; viable cells in all groups were all PgP-1$^+$ (positive control for stainings). The experiments carried out with LyD9, C4-95/16, and FTD5 clones showed the same results and they are not included above for the sake of simplicity. NP, not possible. (These conditions did not maintain viable cells; hence, it was not possible to test them for differentiating activity on these clones.) In experiments of group B, the progenitor clones were cultured with RP.0.10 stroma cells, LPS, and either rIL-7 or limiting amounts of rIL-3. In experiments of group C, the cultures were established under conditions that were either permissive for cell interactions between the pro-B cells and the RP.0.10 stroma cells or prevented such cell interactions [by using transwell Costar plates equipped with filter membrane separating the pro-B cells (on top) and the RP.0.10 stroma cells + LPS + rIL-7 (at the bottom)]. Numbers indicate percent positive cells. The range of positive cells detected in three experiments (group B) and two experiments (group C) is shown.

Table 2 (group B) summarizes the results obtained in the three experiments carried out.

The next set of experiments was aimed at testing whether cell interactions between the pro-B cell and the RP.0.10 stroma cell were required or if soluble factors produced by RP.0.10 stroma cells in the presence of rIL-7 and LPS were sufficient to induce their differentiation into IgM$^+$ B cells. Thus, cultures in which monolayers of RP.0.10 stroma cells, rIL-7, and LPS contained in the bottom of the Costar transwells were separated from the pro-B cells by a membrane filter were established. Cultures in which the pro-B cells could interact with RP.0.10 stroma cells (in the absence of the membrane filter) were also set up in parallel and served as positive controls. The results obtained in the two experiments carried out are shown in Table 2 (group C). No IgM$^+$ B cells were detected in cultures in which cell–cell contact between pro-B cells and RP.0.10 stroma cells was precluded, while significant numbers of IgM$^+$ B cells were generated in the cultures that were permissive for pro-B cell–stroma cell interactions. The data indicate that putative soluble factors produced by RP.0.10 stroma cells cultured in the presence of rIL-7 and LPS cannot induce differentiation of the pro-B clones LyB9 and CB/Bm7 into IgM$^+$ B lymphocytes. Consistent with it, previously we could not replace the differentiating property of RP.0.10 stroma cells with supernatants obtained from confluent cultures of either RP.0.10 cells or heterogenous marrow stroma cells stimulated with a variety of agents (LPS, phorbol 12-myristate 13-acetate plus ionomycin, and interleukins from rIL-1α to rIL-6) (19). Thus, cell–cell contact between the pro-B cell and the marrow stroma cell must be essential to trigger rearrangement and expression of the immunoglobulin genes in the pro-B clones.

We stress that this conclusion may only apply to progenitor cells having the immunoglobulin genes in the germ-line configuration such as the pro-B clones studied here and in no way do our results argue against the possibility that rIL-7 or other soluble factors could by themselves affect the state of already rearranged immunoglobulin genes at later stages of B-cell development (i.e., pre-B and B cells).

Effect of rIL-7 Alone or in the Presence of RP.0.10 Stroma Cells on Marrow pro-T Clones and Fetal Thymocyte Clones. rIL-7 alone did not stimulate DNA synthesis on the three marrow pro-T clones (C4-77/3, C4-86/18, C4-95/16) or in the three fetal thymocyte clones (FTH5, FTA2, FTD5) tested. The marrow pro-T clones proliferated in rIL-3 and the fetal thymocyte clones grew in rIL-4 as described (refs. 15 and 17; Fig. 1). rIL-7 alone or in various combinations with other cytokines (from rIL-1α to rIL-6) did not induce the pro-T clones to differentiate into T3$^+$ T cells or IgM$^+$ B cells (Table 2, group A). The marrow pro-T clones and the fetal thymocyte clones cocultured with RP.0.10 stroma cells and rIL-7 neither grew nor differentiated into T3$^+$ T cells or IgM$^+$ B cells (group B). Elsewhere, it will be shown that the marrow pro-T clones as well as the fetal thymocyte clones can differentiate *in vitro* into T3$^+$ T cells but not IgM$^+$ B cells when cocultured with thymic epithelial cell lines (R.P., J. Pelkoven, S. Stuber, and J. Samaridis, unpublished data).

The remarkable effects of rIL-7 on growth and differentiation of the pro-B clones observed in the presence of the RP.0.10 stroma clone deserves further discussion. There are several possible mechanisms that could account for it, and

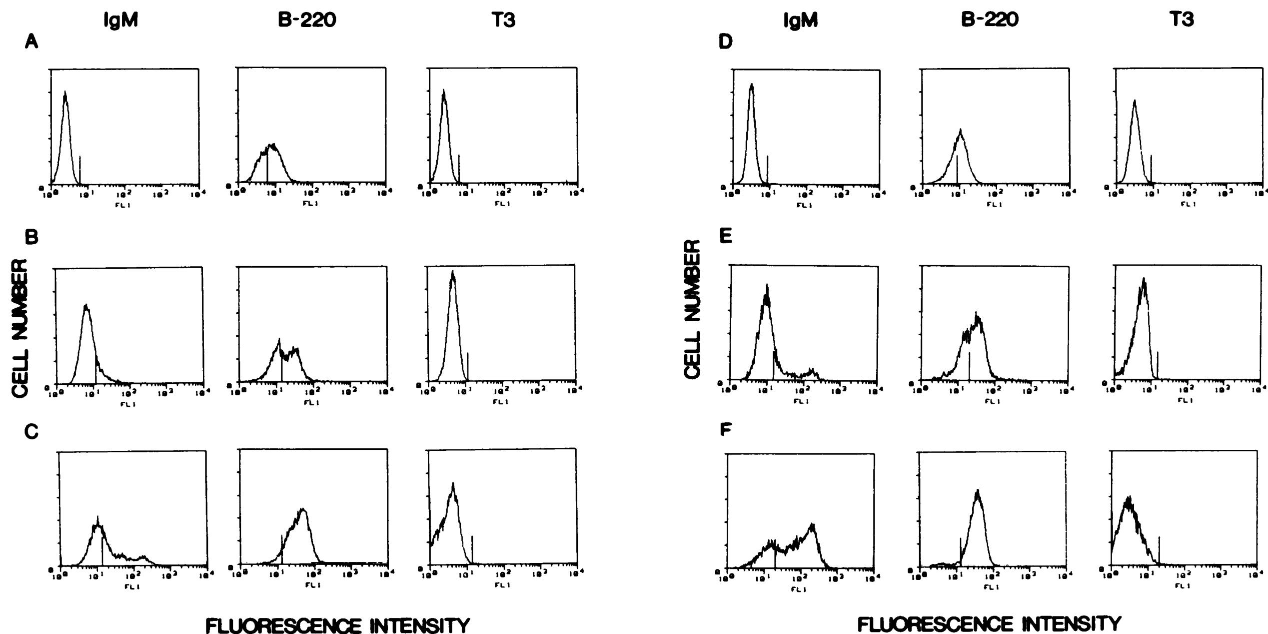

FIG. 2. Differentiation of LyB9 and CB/Bm7 pro-B clones induced by coculture with RP.0.10 stroma cells, LPS, and either rIL-7 or rIL-3. The presence of surface IgM^{+}, B-220^{+}, and $T3^{+}$ cells was assessed by FACS analysis after 10 days of culture. (*A*) Uninduced LyB9 cells. (*B*) LyB9 cells cocultured with RP.0.10 stroma cells, LPS, and rIL-3. (*C*) LyB9 cells cocultured with RP.0.10 stroma cells, LPS, and rIL-7. (*D*) Uninduced CB/Bm7 cells. (*E*) CB/Bm7 cells cocultured with RP.0.10 stroma cells, LPS, and rIL-3. (*F*) CB/Bm7 cells cocultured with RP.0.10 stroma cells, LPS, and rIL-7. Similar results were obtained with the LyD9 pro-B clone (data not shown).

they are by no means mutually exclusive. It is possible that IL-7 bound to the cell membrane of RP.0.10 stroma cells functions much more efficiently than in soluble form as reported for other hemopoietic growth factors (21). Also, IL-7 could stimulate RP.0.10 stroma cells to perform better in inducing differentiation of the pro-B cells. Perhaps the pro-B cells are induced to express higher numbers of IL-7 receptors or the affinity of such receptors for IL-7 increases upon interaction with the RP.0.10 stroma cells. It could also act by rendering the pro-B clones more susceptible to the differentiating stimuli provided by the RP.0.10 stroma cells. In light of the significant numbers of both B-220^{+} IgM^{-} and B-220^{+} IgM^{+} B cells generated in the presence of RP.0.10 stroma cells and IL-7, we ought to entertain the possibility that IL-7 must have also promoted the proliferation and differentiation of less-immature progeny (e.g., pre-B cells) of the pro-B clones as they developed in the cultures. Actually, IL-7 has been identified and purified by its property of supporting proliferation of pre-B cells developed in long-term bone marrow cultures (1, 2). The combination of some or all of these mechanisms could well be operating in this *in vitro* clonal system for pro-B-cell differentiation. Further work, including development of antibodies against the receptors for IL-7, is necessary to determine which of these mechanisms operate in this system.

Unlike the effects of IL-7 on B-cell precursors, we have found no evidence that IL-7 affects growth or differentiation of prethymic and intrathymic T-cell progenitor clones. Since the T-cell progenitor clones studied here contain the T-cell receptor genes in the germ-line configuration, our results cannot exclude the possibility that IL-7 might act on cells at later stages of T-cell development (pre-T and T cells). In light of the results described here, it seems reasonable to postulate that during development both the interaction of the pluripotential stem cell with marrow stroma cells like RP.0.10 and the availability of IL-7 may play a critical role in the commitment to differentiate along the B-lymphocyte pathway.

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Note Added in Proof. We have recently found that rIL-7 promotes DNA synthesis of activated thymocytes from adult mice and peripheral T lymphocytes, indicating that IL-7 may act either directly or indirectly on T-lineage cells at a developmental stage later than pro-T cells.

1. Namen, A. E., Schmierer, A. E., March, C. J., Overell, R. W., Park, L. S., Urdal, D. L. & Mochizuki, D. Y. (1988) *J. Exp. Med.* **167**, 988–1002.
2. Namen, A., Lupton, S., Hjerrild, K., Wignall, J., Mochizuki, D., Shmierer, A., Mosley, B., March, C., Urdal, D., Gillis, S., Cosman, D. & Goodwin, R. (1988) *Nature (London)* **333**, 571–573.
3. Whitlock, C., Tidmarsh, G., Müller-Sieburg, L. & Weissman, I. L. (1987) *Cell* **48**, 1009–1021.
4. Hunt, P., Robertson, D., Weiss, D., Rennick, D., Lee, F. & Witte, O. N. (1987) *Cell* **48**, 997–1007.
5. Kincade, P., Lee, G., Watanabe, T., Sun, L. & Scheid, M. (1981) *J. Immunol.* **127**, 2262–2268.
6. Leo, O., Foo, M., Sachs, D. H., Samelson, L. E. & Bluestone, J. A. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 1374–1378.
7. O'Hara, J. & Paul, W. E. (1985) *Nature (London)* **315**, 333–335.
8. Malek, T., Robb, R. & Shevach, E. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 5694–5699.
9. Ceredig, R., Lowenthal, J., Nahbolz, M. & MacDonald, R. (1985) *Nature (London)* **314**, 98–100.
10. Palacios, R., Neri, T. & Brockhaus, M. (1986) *J. Exp. Med.* **163**, 369–378.
11. Vink, A., Gomlie, P., Wauters, P., Nordan, R. & van Snick, J. (1988) *Eur. J. Immunol.* **18**, 607–612.
12. Karasuyama, H. & Melchers, F. (1988) *Eur. J. Immunol.* **18**, 97–104.
13. Palacios, R., Karasuyama, H. & Rolink, A. (1987) *EMBO J.* **6**, 3687–3693.
14. Palacios, R. & Steinmetz, M. (1985) *Cell* **41**, 727–734.
15. Palacios, R., Kiefer, M., Brockhaus, M., Karjalainen, K., Dembic, Z., Kisielow, P. & von Boehmer, H. (1987) *J. Exp. Med.* **166**, 12–21.
16. Palacios, R. & Pelkonen, J. (1988) *Immunol. Rev.* **104**, 5–27.
17. Pelkonen, J., Sideras, P., Rammensee, H. G., Karjalainen, K. & Palacios, R. (1987) *J. Exp. Med.* **166**, 1245–1258.
18. Pelkonen, J., Tunnacliffe, A. & Palacios, R. (1988) *Eur. J. Immunol.* **18**, 1337–1341.
19. Palacios, R., Stuber, S. & Rolink, A. (1989) *Eur. J. Immunol.*, in press.
20. Kinashi, T., Inaba, K., Tsubata, T., Tashiro, K., Palacios, R. & Honjo, T. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4473–4478.
21. Gordon, M., Ryley, G., Watt, S. & Greaves, M. F. (1987) *Nature (London)* **326**, 403–405.