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Identification of an IL-7-Associated Pre-Pro-B Cell Growth-Stimulating Factor (PPBSF). I. Production of the Non-IL-7 Component by Bone Marrow Stromal Cells from IL-7 Gene-Deleted Mice

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Mouse bone marrow (BM) stromal cell conditioned medium (CM) from our long-term lymphoid culture system selectively induces the in vitro proliferation and presumptive differentiation of pre-pro-B cells (B220⁻, HSA⁻, TdT⁻ or TdT⁺, cμ⁻) from adult rat, mouse, and human BM. However, the responsible growth factor(s) has not yet been identified. Inasmuch as IL-7 is one of the cytokines most closely associated with early B-lineage development, we utilized BM adherent cells and stromal cell lines from IL-7 gene-deleted (−/−) mice in combination with rIL-7 and anti-IL-7 mAb to investigate its possible regulatory role in our culture system. The results show that, although rIL-7 and IL-7 (−/−) CM each can maintain the viability of freshly harvested pre-pro-B cells in vitro, neither induces them to proliferate and/or differentiate, even in the presence of recombinant stem cell factor (rSCF) and/or recombinant insulin-like growth factor (rIGF). The results also show that anti-IL-7 mAb fails to neutralize the pre-pro-B cell growth-stimulating activity in IL-7 (+/+) CM. Yet rIL-7 enables IL-7 (−/−) CM to induce proliferation of pre-pro-B cells, and to “prime” them to respond directly to monomeric IL-7. Furthermore, anti-IL-7 mAb adsorbs the pre-pro-B cell growth-stimulating activity from both IL-7 (+/+) CM and IL-7-supplemented IL-7 (−/−) CM; but rIL-7 does not restore this activity. Lastly, both pre-pro-B cell growth-stimulatory activity and IL-7 are quantitatively recovered by ultrafiltration in the 50 to 100 kDa, rather than the 10 to 50 kDa, apparent molecular mass fraction. These results suggest that the pre-pro-B cell growth-stimulating activity in our culture system is the property of a self-associating complex of IL-7 and a second BM stromal cell-derived cofactor.

In the present study, the results of in vitro Ab neutralization, immunoadsorption, and cytokine reconstitution experiments, combined with preliminary m.w. determination, suggest that the pre-pro-B cell growth-stimulating factor (PPBSF) in CM from our culture system (21) is a bimolecular complex of IL-7 and an as yet unidentified cofactor, the latter of which is produced by BM stromal cells from IL-7 (-/-) mice. These results are confirmed and extended by Western immunoblotting in a companion paper (23). Unlike IL-7, PPBSF is resistant to neutralization with anti-IL-7 mAb and does not induce proliferation of pre-B cells or pre-B cells. Rather, PPBSF selectively stimulates proliferation and (presumably) differentiation of pre-pro-B cells, which normally are adherent to BM stromal cells (24, 25). In addition, PPBSF appears to “prime” pre-pro-B cells to respond to monomeric IL-7 in an adhesion-independent fashion. Hence, as discussed, differences in molecular form, receptor affinity, and, possibly, site of expression may enable IL-7 to regulate early B-lineage development in a stage-specific manner.

Materials and Methods

Animals

Male 4- to 6-wk-old IL-7 gene-deleted (IL-7 (-/-)) and nondeleted (IL-7 (+/+)) mice (13), bred from (129 X B6 F1) stock generously provided by Drs. Richard Murray and Ursula von Freeden-Jeffry (DNAX Research Institute of Cellular and Molecular Biology, Palo Alto, CA), were used as donors of BM-adherent cells and stromal cell lines. Male 4- to 6-wk-old Lewis strain rats, bred from stock originally obtained from the National Cancer Institute, National Institutes of Health, were used as donors of BM lymphoid precursor cells. Animals were maintained on standard chow and water ad libitum in the Center for Laboratory Animal Care, the University of Connecticut Health Center.

Cytokines and Abs

Recombinant IL-7, SCF, IG-1, and neutralizing mAbs cross-reactive with human and mouse IL-7 were purchased from Genzyme Corporation (Cambridge, MA). Mouse IgG1 isotype control was obtained from Sigma Immunochemicals, St. Louis, MO. Murine mAb to the HIS40 (anti-IgM) (26), human and mouse IL-7 were purchased from Genzyme Corporation (Cambridge, MA), and mouse IL-7 was a generous gift from Drs. Richard Murray and Ursula von Freeden-Jeffry (DNAX Research Institute of Cellular and Molecular Biology, Palo Alto, CA). 12/10 anti-IgG F(ab')(2) fragments were obtained from Kirkegaard and Perry Laboratories, Gaithersburg, MD. Affinity-purified rabbit Ab to calf thymus TdT, and developed with FITC- or TRITC-
microfluor (27) or rat primary Abs (10 

Immunofluorescence

Staining viable cells in suspension with the appropriate Abs and then staining cytocentrifuge smears of the same cells for BrdU. Labeled cells were quantified using a Zeiss Universal fluorescence microscope equipped with narrow band filters for FITC and TRITC or PE.

Lymphoid culture system

Rat BM pre-pro-B cells and pro-B cells were generated in our culture system as previously described (16). Briefly, single cell suspensions of mouse BM (8 X 10^6 cells) were added to 2 ml RPMI 1640 containing 20% FBS selected, defined FBS (HyClone, Logan, UT) in 35-mm diameter culture plate wells and incubated at 37°C in 5% CO₂. After 10 days, the confluent adherent cell layers were washed and seeded with 5 X 10^5 freshly harvested rat BM cells/ml. In some experiments, the rat BM cells were seeded into microporous membrane culture inserts (0.4 μm pore size; Transwell-3408, Costar, Cambridge, MA) placed over (but not in contact with) the mouse BM-adherent cell layers. In experiments in which the cultures were treated with Ab, the cultures were incubated with anti-IgM mAb or rat BM cells. Total cells from the culture inserts and nonadherent lymphoid cells from the standard cultures were recovered in serum-free medium on day 10 for cytologic and phenotypic analysis or for transfer to secondary cultures (21).

Establishment of mouse BM stromal cell lines

Day 10 primary BM adherent cell layers from IL-7 (+/+) or IL-7 (-/-) mice grown in RPMI 1640 with 20% FBS were detached with 0.05% trypsin 0.53% EDTA 4Na (Life Technologies, Grand Island, NY) and dissociated by gentle pipetting. The suspended cells were plated in a 25-cm² flask and grown to confluency. The cells were repeatedly passaged at 3- to 4-day intervals for approximately 2 mo to generate morphologically homogeneous stromal cell lines, as described (21).

Conditioned medium (CM)

Washed confluent mouse BM-adherent cell layers or stromal cell lines therefrom were used to condition medium for 10 days (21). The CM for cell stimulation was filtered to remove any cells, concentrated twofold by ultrafiltration in Centriprep-10 Concentrator units (Amicon, Danvers, MA), dialyzed for 16 h in serum-free normal medium at 4°C, and stored at -70°C. For cell stimulation, CM was diluted to twofold its original concentration with medium containing 20% FBS; for immunoadsorption, 10X concentrated CM in serum-free normal medium was used; for semiquantitative dot blot analysis for IL-7, serum-free CM was collected after 4 days incubation and concentrated 10-fold.

Fractionation of CM using size exclusion membranes

Serum-free CM was concentrated 20-fold in a series of Amicon filters with graded MW cutoffs as follows: CM was concentrated in a stirred cell filtration unit using a YM-100 55-mm preosmed membrane. Retentate was kept as the >100-kDa molecular mass fraction and filtrate was sequentially concentrated using an XM-50 membrane (to obtain a 50- to 100-kDa molecular mass fraction), Centriprep-30 (30- to 50-kDa molecular mass fraction), and Centriprep-10 (10- to 30-kDa molecular mass fraction).

Immunoadsorption of CM with anti-IL-7 mAb

Anti-IL-7 mAb (mouse IgG₁; Ab conjugated to Protein A-Sepharose by incubating 15 μl of Ab with 80 μl of packed beads for 4 h. The beads were extensively washed with PBS to remove unbound Ab. Immunoadsorption was accomplished by incubating 10X concentrated CM with Ab-conjugated Protein A-Sepharose beads (1 ml CM/80 μl packed beads) in a rotating mixer for 2 h at 4°C. The beads were pelleted in a microfuge (8,000 r.p.m.) and the supernatant was removed. This process was repeated 3 times. Nonspecific binding was controlled by incubating CM with unconjugated Protein A-beads and with beads conjugated with a mouse IgG₂ isotype control. Adsorbed CM was then filter sterilized, and assayed for residual IL-7 by semiquantitative dot blot analysis and thymocyte proliferation analysis, respectively. The bound Ab was recovered from the beads by elution with 0.1 M NaHCO₃ buffer (pH 9.3) containing 0.5 M NaCl, and the eluate was dialyzed for 16 h in PBS (pH 7.2) at 4°C.

Dot immunoblotting of CM for IL-7

Immobilon-P membranes (Millipore, Bedford, MA), preosmed in Tris-Glycine Buffer with 20% ethanol, received 5 to 10 μl of adsorbate or eluate per sample of CM by micropipet. Standards of serially diluted rIL-7 were added to each sample of CM by micropipet. Standards of serially diluted rIL-7 were added to each sample of CM by micropipet.
FIGURE 1. Ability of BM-adherent cells and CM from IL-7 (+/+) and IL-7 (−/−) mice to support lymphopoiesis in pro-B-type cultures of rat BM; effect of rIL-7. Triplicate wells containing confluent layers of IL-7 (+/+) or IL-7 (−/−) BM-adherent cells plus or minus rIL-7 (5 ng/ml) were inoculated with 5 × 10^5 freshly harvested rat BM cells/ml. The numbers of B-lineage cells/well in the nonadherent compartment were determined on day 10 as follows: total (B220^+); pre-pro-B (B220^+ HSA<sup>c+</sup> c<sub>M</sub>^−<sub>−</sub>); pro-B (B220^+ HSA<sup>c+</sup> c<sub>M</sub>^−^<sub>−</sub>); pre-B (B220^+ HSA<sup>c+</sup> c<sub>M</sub>^−^<sub>−</sub>); and B (B220^+ HSA<sup>c+</sup> sIgM<sup>+</sup>). Results represent the means of a representative experiment (one of four). The number of sIgM<sup>+</sup> B cells is not indicated since these were seen only in the input inoculum.

FIGURE 2. Ability of IL-7 (+/+) and IL-7 (−/−) CM to maintain or expand lymphoid progenitor cell activity in pro-B-type cultures of rat BM: effect of rIL-7. Quadruplicate wells of IL-7 (+/+) CM or IL-7 (−/−) CM were inoculated with 5 × 10^5 freshly harvested rat BM cells/ml in the presence or absence of rIL-7 (5 ng/ml). On day 4, the cells from each group of wells were pooled, serially twofold diluted in normal medium (NM), and passaged on a per well equivalency basis onto IL-7 (+/+) mouse BM-adherent cell layers in standard pro-B-type cultures for an additional 10 days. The mean numbers of lymphoid cells in these secondary (2°) cultures were then compared with those generated in standard primary (1°) cultures of serially twofold diluted freshly harvested rat BM cells. Results represent the means of duplicate cultures. No lymphoid cells were generated in secondary cultures after primary incubation of BM in normal culture medium.

**Results**

**IL-7 is required to generate pre-pro-B cells and pro-B cells in vitro**

To examine the possible role of IL-7 in our culture system, rat BM cells were incubated for 10 days on BM-adherent cell layers from IL-7 (+/+) and IL-7 (−/−) mice. Results in Figure 1 show that IL-7 (+/+) BM-adherent cells preferentially stimulated the expansion of pre-pro-B cells and pro-B cells, whereas IL-7 (−/−) BM-adherent cells maintained input numbers only of pre-pro-B cells. However, rIL-7 (5 ng/ml) not only enhanced lymphopoiesis in cultures containing IL-7 (+/+) BM-adherent cells, but also enabled cultures containing IL-7 (−/−) BM-adherent cells to expand the pool of pre-pro-B cells and to generate pro-B cells. In contrast, rIL-7 alone was able to maintain input numbers only of pre-pro-B cells, even at higher concentrations of rIL-7 (10–100 ng/ml) alone or in the presence of rSCF (10–500 ng/ml) and/or rIGF (4–80 ng/ml) (data not shown).

To quantify and further define the effects of the soluble products of mouse BM-adherent cells on the maintenance and/or expansion of lymphoid precursor cell activity, rat BM cells were incubated for 4 days in IL-7 (+/+) or IL-7 (−/−) CM; and the surviving cells were serially twofold diluted, passaged onto BM-adherent cells from IL-7 (+/+) mice, and cultured for an additional 10 days. Results in Figure 2 show that rIL-7 in normal medium (B) and IL-7 (−/−) CM (C) each maintained input levels of lymphoid precursor activity (A), whereas a combination of rIL-7 and IL-7 (−/−) CM (D) or IL-7 (+/+) CM alone (E) expanded this activity four- to eightfold above input levels. Even greater precursor activity was detected among the cells from primary cultures that contained both rIL-7 and IL-7 (+/+) CM (F).

These observations were repeated with CM produced by established lines of BM stromal cells from IL-7 (+/+) and IL-7 (−/−) mice (data not shown, but see Ref. 21).

**Anti-IL-7 mAb adsorbs, but does not neutralize, the pre-pro-B cell growth-stimulating activity in IL-7 (+/+) CM**

As shown in Figures 3 and 4, respectively, almost all of the growth-stimulating activity for pro-pre-B cells was removed from 10-fold concentrated IL-7 (+/+) CM by adsorption with anti-IL-7 mAb bound to Protein A-Sepharose, and this activity was quantitatively recovered by elution under high salt concentration. Nonetheless, the pre-pro-B cell growth-stimulating activity of the anti-IL-7 mAb-adsorbed CM was not reconstituted by addition of rIL-7 (Fig. 3). The latter result was not due to the action of residual anti-IL-7 mAb, inasmuch as rIL-7 fully reconstituted the thymocyte growth-stimulating activity of the adsorbed CM (data not shown). Hence, adsorption of IL-7 (+/+) CM with anti-IL-7 mAb appears to simultaneously remove IL-7 and a physically associated cofactor, which together constitute a pre-pro-B cell growth-stimulating factor (PPBSF).
Medium plus rIL-7 were obtained after treatment with culture-generated rat BM lymphoid cells were incubated in (10-fold concentrated IL-7 (5 ng/ml). Triplicate wells were incubated with 5 × 10⁵ freshly harvested rat BM cells/ml and incubated for 4 days. Results indicate mean numbers of total B-lineage cells (± SD) in 3 experiments. *Denotes value significantly greater (p < 0.05) than that for adsorbed CM, with or without rIL-7. No significant reduction in lymphopoietic activity was observed by sham-adsorption of CM with Protein A-Sepharose beads alone or conjugated with IgG2b isotype control mAb.

Results in Figure 5 show that the pre-pro-B cell growth-stimulating activity in IL-7 (+/+) CM was not neutralized by doses of anti-IL-7 mAb up to 5 times greater than that required to completely inhibit the activity of 5 ng/ml rIL-7 added to either normal medium or IL-7 (+/+) CM. Yet, as shown in Figure 6, lymphopoiesis could be inhibited by addition of anti-IL-7 mAb to cultures of rat BM cells placed directly on, or separated by, a microporous membrane culture insert from IL-7 (+/+ ) mouse BM-adherent cells. These observations suggest that, under these circumstances, IL-7 on the stromal cell surface (or recently released into the medium) is neutralized prior to the formation of the PPBSF complex.

rIL-7 forms a biologically active molecular complex with a cofactor in IL-7 (−/−) CM

To test our working hypothesis that PPBSF is a self-associating molecular complex of IL-7 and a second, stromal-cell derived, growth factor, rIL-7 was added to IL-7 (−/−) CM before and/or after adsorption with anti-IL-7 mAb. As shown in Figure 7, A and B, the ability of rIL-7 to stimulate thymocyte proliferation when added to normal medium or IL-7 (+/+) CM was neutralized by anti-IL-7 mAb. In contrast, the ability of rIL-7 to stimulate thymocyte proliferation when added to IL-7 (−/−) CM was not neutralized by the subsequent addition of anti-IL-7 mAb. Furthermore, as shown in Figure 7C, addition of rIL-7 to IL-7 (+/+ ) CM and IL-7 (−/−) CM after adsorption with anti-IL-7 mAb, enabled both to stimulate thymocyte proliferation. However, only the activity in the IL-7 (+/+ ) CM could be neutralized with anti-IL-7 mAb. Conversely, addition of rIL-7 to IL-7 (−/−) CM prior to adsorption with anti-IL-7 mAb enabled rIL-7 to restore the thymocyte-stimulatory activity in a neutralizable manner.

Similarly, results in Figure 8A show that immunoadsorbed IL-7 (−/−) CM to which rIL-7 had not been added initially was able to stimulate proliferation of pre-pro-B cells in a non-neutralizable...
manner after subsequent addition of rIL-7. However, rIL-7 was unable to restore PPBSF activity to immunoadsorbed IL-7 (2/2) CM to which rIL-7 had been added initially (Fig. 8B). As anticipated (see Figs. 3 and 4), PPBSF activity was quantitatively recovered in the eluate (data not shown).

Hence, rIL-7 appears to complex spontaneously with a soluble factor in IL-7 (2/2) CM to form a functional PPBSF, similar to that which normally exists in IL-7 (1/1) CM. In both instances, PPBSF has thymocyte and pre-pro-B cell growth-stimulating activities, and is bound, but not neutralized, by anti-IL-7 mAb.

Apparent molecular mass of PPBSF

The approximate molecular mass of PPBSF in IL-7 (1/1) CM was determined by ultrafiltration using a series of membranes with graded molecular mass exclusion sizes. These fractions were then tested for their ability to support the growth of pre-pro-B cells and pro-B cells in vitro. Virtually all of the PPBSF activity in the CM was recovered in the 50- to 100-kDa apparent molecular mass fractions (Table I). Similarly, all detectable IL-7 (nominal molecular mass 25 kDa) was recovered in this fraction, as determined by semiquantitative dot blot analysis (data not shown). These results further support the notion that PPBSF is a molecular complex of IL-7 and an associated cofactor.

PPBSF “primes” pre-pro-B cells to respond to rIL-7 alone

To more precisely define the role of PPBSF in the development of early B-lineage cells, freshly harvested rat BM cells were incubated for 4 days in IL-7 (1/1) CM or IL-7 (2/2) CM plus rIL-7, after which the cells were transferred into normal medium plus rIL-7 for an additional 4 days. Results in Figure 9 demonstrate that rIL-7 was able to stimulate the expansion of both pre-pro-B cells and pro-B cells in secondary cultures of BM lymphoid cells that had first been exposed to PPBSF; and simultaneous analysis of BrdU incorporation and antigenic phenotype in these secondary
cultures revealed that both pro-B cells and pre-pro-B cells were proliferating (data not shown). This is in contrast to results obtained with the primary cultures, in which only pre-pro-B cells proliferated (also see ref. (21).

Discussion

The present results demonstrate that IL-7 can maintain the viability of pre-pro-B cells in vitro but suggest that an additional BM stromal cell-derived cofactor, acting in concert with IL-7, is required to stimulate their proliferation and to "prime" them to respond to IL-7 alone. These observations are consistent with those of other investigators (6, 8–11, 31–35) who have postulated that factors other than (or in addition to) IL-7 are required to stimulate expansion of mouse or human presumptive pre-pro-B cells in vitro or in vivo. Hence, we reasoned that, if the cofactor in our culture system existed independently of IL-7, it should be detectable functionally by reconstituting IL-7-deficient CM with rIL-7. This in fact occurred. However, PPBSF activity was not detected when rIL-7 was added to IL-7 (−/−) CM that had first been adsorbed with anti-IL-7 mAb in the presence of rIL-7; neither was it detected when rIL-7 was added to IL-7 (+/+) CM that had been adsorbed with anti-IL-7 mAb. Thus, by analogy with other lymphoid growth factors (e.g., 36–38), PPBSF appeared to be a molecular complex of IL-7 and a second, independently regulated, growth-factor. Under these circumstances, adsorption with anti-IL-7 mAb would eliminate PPBSF activity from IL-7 (+/+) CM and rIL-7-supplemented IL-7 (−/−) CM by simultaneously removing both IL-7 and the antigenically unrelated cofactor. This hypothesis was corroborated by 1) quantitative recovery of PPBSF activity from the anti-IL-7 mAb immunoabsorbent beads; 2) recovery of both PPBSF activity and IL-7 in the 50- to 100-kDa apparent molecular mass ultrafiltrate fraction; and 3) failure of anti-IL-7 mAb to neutralize the IL-7 activity in CM.

Although it might be argued that sufficient IL-7 is produced by the rat BM cell inoculum itself to mask the neutralizing effect of anti-IL-7 mAb, this does not appear to be the case. Anti-IL-7 mAb-adsorbed IL-7 (+/+) CM fails to support BM lymphopoiesis when reconstituted with rIL-7, and anti-IL-7 mAb neutralizes the activity of rIL-7 added to IL-7 (+/+) CM. Also, rat BM-adherent cells do not support pro-B cell development in our culture system unless supplemented with rIL-7 (16, 21; and our unpublished observations).

Additional evidence that PPBSF is a covalently linked, IL-7-associated heterodimer is provided in a companion manuscript (23). Therefore, only the developmental implications of the present observations are discussed here. Specifically, the question arises as to why a specialized form of IL-7 selectively supports the growth of pre-pro-B cells in vitro. Two possibilities are manifest based on properties inherent to pre-pro-B cells. The first relates to the need for cognate interactions between pre-pro-B cells and BM stromal cells for optimal lymphopoiesis; the second reflects the need of pre-pro-B cells to self-replicate to maintain the precursor cell pool (2, 3, 21, 24, 25, 39). The ability of PPBSF to satisfy both requirements may help to explain why our culture system is able to generate pre-pro-B cells indeﬁnitely (16, 21, 24). Thus, the fact that neat CM is only about 10% as effective as are BM stromal cells in supporting the growth of pre-pro-B cells (21, 24) suggests, as one possibility, that PPBSF functions primarily as a membrane (or extracellular matrix)-bound complex. Similarly, continued expansion of the pool of pre-pro-B cells in the absence of pluripotent stem cells suggests that PPBSF can induce proliferation without differentiation (24).

It is more difﬁcult at this point to assess the role of PPBSF in inducing differentiation of pre-pro-B cells, even though most pro-B cells in our culture system appear to originate from such precursors (21, 24, 25, 40). At a minimum, PPBSF must be indirectly involved, since neither IL-7 nor the PPBSF co-factor induces the appearance or survival of pro-B cells. However, it is possible that PPBSF merely "primes" pre-pro-B cells to differentiate (as well as to proliferate) in response to subsequent stimulation with trace amounts of monomeric IL-7. The answer to this question should be forthcoming from ongoing experiments using puriﬁed PPBSF and "early" pre-pro-B cells (B220+; HSA−; TdT−; cμ−).
Regardless of the result, a plausible scenario would that permit different forms of IL-7 to provide developmental continuity between pre-pro-B cells and pro-B cells envisions stage-specific differences in the expression of the IL-7R. Given that IL-7Ry chain gene-deleted mice generate both pre-pro-B cells and pro-B cells (41, 42), whereas IL-7Rα-chain gene-deleted mice appear to generate pre-pro-B cells only (12), it is possible that PPBSF is designed to transmit a proliferative signal involving low affinity (or otherwise altered) forms of the IL-7R. Conversely, monomeric IL-7 may require high affinity IL-7R for efficient signal transduction (43–46). Hence, PPBSF may selectively regulate the G/β/δ transition of pre-pro-B cells (21), much as monomeric IL-7 selectively regulates the G/β/δ transition of pro-B cells (47).

The postulated sequential expression of low and high affinity IL-7R during early B-lineage development is analogous to events observed during early thymocyte development (48, 49). It is supported by the reported difficulty in detecting expression of high affinity IL-7R on pre-pro-B cells, but not pro-B cells (50). It is also consistent with the failure of an excess of IL-7 (51, 52) and a deficit of high affinity IL-7R (12, 53), respectively, to increase or decrease the generation of pre-pro-B cells in vivo. Induction of high affinity (or other forms or sites densities of) IL-7R on activated pre-pro-B cells would also help to explain the “priming” effect of PPBSF for monomeric IL-7R. In turn, the sequential actions of PPBSF and monomeric IL-7 would correlate nicely with the demonstration of separate microanatomical niches (24, 25, 54), differentiative events in the expression of the IL-7R. Given that IL-7R during early B-lineage development is analogous to events explained by mouse bone marrow stromal cells. It might also be argued that PPBSF can bind a receptor other than (or in addition to) the IL-7R. Similar explanations may apply to conflicting reports regarding the need for IL-7 in normal human B cell ontogeny (14, 59, 60), although important species-specific differences may exist. The availability of purified PPBSF cofactor and Abs thereto (23) may help to clarify these issues.

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References


