Modulation of the IL-7 Dose-Response Threshold During Pro-B Cell Differentiation Is Dependent on Pre-B Cell Receptor Expression

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The IL-7R and the pre-B cell receptor (pre-BCR) each provide critical signals during differentiation of B cell precursors. In this study we examine the interplay between signals dependent upon these receptors. We demonstrate that pre-BCR-deficient pro-B cells differ significantly from controls in their ability to use the IL-7R. We show that this difference, characterized by a failure to proliferate in response to IL-7, is narrowly restricted to IL-7 concentrations in the picogram per milliliter range and can be overcome with increasing amounts of IL-7. Restoration of Ig heavy chain to recombinase-activating gene-2-deficient pro-B cells leads to a restored response to picogram per milliliter levels of IL-7, providing strong evidence that modulation of the IL-7 dose-response threshold is dependent on pre-BCR. Culture of normal pro-B cells under low IL-7 conditions leads to selective outgrowth of cells expressing μ heavy chain, suggesting that modulation of IL-7 dose-response thresholds can allow for selective expansion of pre-BCR+ cells under conditions where IL-7 is limiting. We also provide evidence that expression of pre-BCR on pro-B cells limits the duration of IL-7 responsiveness by causing differentiation to an IL-7-unresponsive pre-B cell stage. Thus, the pre-BCR-dependent modulation of IL-7 responsiveness affects both the dose-response threshold and the duration of IL-7-induced clonal expansion. Our results suggest that positive selection of pre-BCR+ pro-B cells may be achieved through the fine tuning of IL-7 responses. The Journal of Immunology, 1998, 161: 6038–6045.

Differential of B cell precursors in the primary lymphoid tissues can be divided into a series of stages based on changes in Ig gene rearrangement status and expression of specific protein markers (1–6). Ig heavy chain (HC) gene rearrangement occurs during the pro-B cell stage, which is characterized by large cell size, expression of terminal deoxynucleotidyl transferase (TdT), expression of the surface Ag CD43, and absence of the surface Ags CD2 and CD25. The majority of Ig light chain (LC) gene rearrangements occur at the pre-B cell stage, which is characterized by small cell size, expression of CD2 and CD25, and absence of terminal deoxynucleotidyl transferase (TdT) and CD43 expression. After a productive LC rearrangement is made, IgM can be expressed on cell surface, which is a defining marker of the immature B cell stage. Immature B cells undergo further differentiation events to become IgM+IgD+ mature B cells.

Pro-B cells generating a productive HC gene rearrangement can express an early form of the Ig receptor, termed the pre-B cell receptor (pre-BCR), which contains the μ HC, the surrogate light chain proteins λ5 and VpreB, the signaling components Ig-α and Ig-β, and several unidentified glycoproteins (7–9). Several lines of evidence have indicated that pre-B cells that make a functional HC gene rearrangement and express pre-BCR are triggered to enter a stage of rapid cell division before differentiating into quiescent small pre-B cells (1, 3, 10, 11). The importance of pre-BCR in regulating the pro-B to pre-B cell transition is demonstrated by studies of mice deficient in expression of pre-BCR components. Mice deficient in expression of HC as well as mice deficient in expression of λ5 show arrest of B cell development at the pro-B cell stage (3, 12, 13). Mice able to produce cytoplasmic but not membrane-bound HCs (14) show a similar arrest in B cell development (3). Expression of Ig HC transgenes in recombinase-activating gene (RAG)-deficient mice restores differentiation to the pre-B cell stage (5, 15), and this restoration requires the presence of λ5 (16). Studies using mutant HC transgenes demonstrate that association of the HC with Ig-α and Ig-β is necessary for promoting the pro-B to pre-B cell transition in RAG-deficient mice (17, 18). Thus, while the critical importance of pre-BCR signals is well established, the mechanisms through which these signals promote B cell differentiation are not understood.

Cytokine receptors are also involved in regulating the B cell developmental cascade. B cell progenitors in the bone marrow are in intimate contact with the processes of reticular stromal cells (1), and such stromal cells have been shown to promote survival and differentiation of B cell progenitors in vitro (19–23). Several cytokines produced by stromal cells have been implicated in the positive or negative regulation of early B cell development. Of these, IL-7 (24) is unique in its ability to induce clonal proliferation of primary B cell progenitors in vitro (22, 25, 26). The importance of IL-7 in B cell development in vivo was underscored by studies examining gene-targeted mice deficient in IL-7 or IL-7R (27, 28). In these studies, loss of the IL-7–IL-7R interaction led to a severe impairment of development beyond the pro-B cell stage. IL-7R is expressed on both pro- and pre-B cells, but not on slgM+ B cells (29–31).
In this study we examine the interplay between pre-BCR and IL-7R signals at the level of biologic responses of primary pro-B cells. Our results provide clear evidence that responses through the IL-7R are modulated during pro-B cell differentiation in a pre-BCR-dependent fashion. Our results suggest that fine tuning of IL-7 responses may provide a mechanism for positive selection of pro-BCR+ pro-B cells.

Materials and Methods

Mice

μMT mice (32) were generated in the laboratory of Dr. K. Rajewsky (Institute for Genetics, Cologne, Germany) and were obtained through Dr. L. Shultz (The Jackson Laboratory, Bar Harbor, ME). RAG-2−/− (13) and RAG-2−/−;HC186 transgenic (tg) (5) mice were provided by Dr. F. Alt (The Children’s Hospital, Boston, MA). RAG-2−/− mice were generated by crossing RAG-2−/− mice with C57BL/6 mice. C57BL/6 mice were purchased from The Jackson Laboratory. All mice were bred and maintained at the Wellesley Hospital Research Institute animal facility (Toronto, Canada) and were used between 6 and 10 wk of age.

Cell purification and culture

Bone marrow cell suspensions were prepared and resuspended at 2 × 10^7 cells/ml in PBS and 5% FCS. Fc receptors were blocked by incubation with the FcBLOCK Ab (PharMingen, San Diego, CA). Cells were then stained with the following labeled Abs (all from PharMingen): FITC-labeled anti-κ light chain (mAb R8-140), phycoerythrin (PE)-labeled anti-CD43 (mAb S7), and biotin-labeled anti-CD19 (mAb 1D3). Staining with the biotin-labeled Ab was revealed by second-step staining with quantum red-streptavidin (Sigma, St. Louis, MO). Cell sorting was performed using a FACSstar Plus (Becton Dickinson, Mountain View, CA) using gates similar to those indicated in Fig. 1. Sorted populations were routinely >95% pure.

Cells were cultured in OPTI-MEM medium (Life Technologies, Gaithersburg, MD) supplemented with 10% FCS, 5 × 10−5 M 2-ME, 100 U/ml penicillin, and 100 U/ml streptomycin (Life Technologies). For limiting dilution experiments this medium was supplemented with 50–100 U/ml murine IL-7 from the supernatant of stably transfected J558 cells (gift from Dr. Ana Cumano, Pasteur Institute, Paris, France). For proliferation assays and bulk culture experiments, purified recombinant mouse IL-7 (R&D Systems, Minneapolis, MN) was added at the indicated concentration at the initiation of culture and was replenished to the same level every 3 days.

In this study we examined the interplay between pre-BCR and IL-7R signals at the level of biologic responses of primary pro-B cells. Our results provide clear evidence that responses through the IL-7R are modulated during pro-B cell differentiation in a pre-BCR-dependent fashion. Our results suggest that fine tuning of IL-7 responses may provide a mechanism for positive selection of pro-BCR+ pro-B cells.

Results

Two different HC-deficient mouse strains were used to analyze B cell development in the absence of pre-BCR: μMT, which can express μ HC in the plasmablast but not the cell surface (32), and RAG-2 deficient, which completely fails to express HC due to absence of Ig gene recombination (13). To examine the interplay between pre-BCR and IL-7R signals, we purified CD19+κ light chain−CD43+ pro-B cells from normal or HC-deficient bone marrow (Fig. 1) and assessed the ability of these cells to proliferate and differentiate in response to IL-7.

HC-deficient pro-B cells have a specific defect in low threshold IL-7 responses

Since the mechanism leading to selective expansion of pre-BCR+ pro-B cells is not known, we hypothesized that pre-BCR-dependent signals might be required for pro-B cells to undergo proliferative responses in the presence of physiologic levels of IL-7. We thus compared the ability of pro-B cells from normal and HC-deficient mice to proliferate in the presence of a wide range of IL-7 concentrations (Fig. 2). Strikingly, we found that HC-deficient pro-B cells exhibited a specific deficiency in the ability to respond to low levels of IL-7. This effect was most pronounced in the picogram per milliliter range of IL-7 concentrations, where the HC-deficient cells respond with up to 10-fold lower levels of proliferation than normal pro-B cells. In contrast, HC-deficient pro-B cells proliferate to an equal or greater extent than normal pro-B cells in the nanogram per milliliter range of IL-7 concentrations (Fig. 2).

The IL-7-responsive cells in these HC-deficient cultures expressed the B-lineage-specific markers CD19, B220 (>95% positive), and BP1 (>80% positive; data not shown), confirming their B-lineage identity. Thus, the deficiency in IL-7 responsiveness exhibited by HC-deficient pro-B cells is not a complete loss of IL-7 responsiveness, but, rather, represents a shift in the IL-7 dose-response threshold.

Abs and cell sorting procedures

For determination of cell surface and cytoplasmic expression of μ HC, cells cultured under the indicated conditions were surface stained with biotin-labeled anti-IgM (mAb 33–60), followed by quantum red-streptavidin (Sigma). Cells were then fixed in 1% paraformaldehyde for 20 min at room temperature. After washing, cells were permeabilized with 0.2% Tween-20 in PBS for 15 min at room temperature. FITC-labeled goat antimouse μ-chain Ab (Jackson ImmunoResearch Laboratories, West Grove, PA) were then added, and the cells were incubated on ice for 20 min. Cells were washed twice with PBS containing 2.5% FCS and 0.2% Tween-20 and then analyzed on a FACSscan flow cytometer (Becton Dickinson). For the experiment shown in Fig. 2, cells were doubly stained with PE-labeled anti-CD2 (mAb RM2–5; PharMingen) and FITC-labeled anti-IgM (mAb 33–60), and the CD2+ IgM+ and CD2− IgM− cells were sorted on the FACStar Plus (Becton Dickinson).

For determination of IL-7R α-chain expression, bone marrow cells were stained for four-color FACS analysis using allophycocyanin-labeled anti-B220 (mAb RA3–6B2; PharMingen), PE-labeled anti-CD43, biotin-labeled anti-IL-7R α-chain (mAb A7R34; gift from Dr. S. Nishikawa), and either FITC-labeled anti-CD19 (mAb ID3; PharMingen) or FITC-labeled anti-κ light chain. Staining with the biotin-labeled Ab was revealed by second-step staining with quantum red-streptavidin. Four-color-stained cells were analyzed on a FACS Calibur flow cytometer equipped with CellQuest software (Becton Dickinson).

CD19+ Bone Marrow

FIGURE 1. Cell sorting gates used to purify pro-B and pre-B cell populations. Erythrocyte-depleted bone marrow cells from the indicated mouse strains were stained with labeled Abs against CD19, CD43, and κ light chain and analyzed on a FACStarPlus cell sorter. The data shown are gated on CD19+ cells falling within the lymphocyte size gate. The gates used for sorting CD43+κ− pro-B cells and CD43−κ− pre-B cells are indicated.
CD43<sup>1</sup> pre-B cell population from control mice showed no significant proliferation over background at any IL-7 concentration (Fig. 2B), in accord with previous findings (2, 30). Together these results suggest that pre-BCR-expressing pro-B cells undergo a transient modulation of the IL-7 dose-response threshold, followed by a complete loss of IL-7 responsiveness during differentiation to the pre-B cell stage.

Culture of pro-B cells under low IL-7 conditions leads to selective outgrowth of cytoplasmic and surface \( \mu^- \) cells

The differential ability of normal and HC-deficient pro-B cells to respond to picogram per milliliter IL-7 concentrations suggests that IL-7, present at such limiting levels, may represent a critical signal for selective expansion of pro-B cells that successfully rearrange and express one of their HC loci. We thus compared cytoplasmic and surface HC in the cell populations responding to nanogram per milliliter and picogram per milliliter IL-7 concentrations.

Pro-B cells isolated from normal or HC-deficient bone marrow were cultured in bulk in the presence of 24 ng/ml or 24 pg/ml IL-7. After 4 days of culture, cell recoveries for control and HC-deficient pro-B cell cultures were similar at 24 ng/ml IL-7, while at 24 pg/ml approximately 10-fold fewer cells were recovered from HC-deficient cultures than from control cultures (Fig. 3A). Recovered cells were stained with Abs against surface IgM and then fixed, permeabilized, and stained for cytoplasmic \( \mu^- \) expression. The data shown are gated on live cells by forward and side scatter. Similar results were obtained in three independent experiments.
expands μ+ cells, while culture with nanogram per milliliter IL-7 concentrations maintains both μ+ and μ− B cell precursors. These data are consistent with the hypothesis that, under limiting conditions, the IL-7 signal alone can support selective expansion of functionally rearranged B cell precursors.

Enforced expression of pre-BCR in RAG-2−/− pro-B cells leads to alteration of the dose-response threshold and kinetics of IL-7-induced proliferative responses

To examine the effect of enforced pre-BCR expression on pro-B cell IL-7 responses we examined RAG-2−/− pro-B cells carrying a rearranged μ transgene (RAG-2−/− Hc tg) (5). On day 4 of culture, the RAG-2−/− Hc tg cultures showed an approximately two-fold lower level of proliferation than RAG-2−/− cultures in the presence of nanogram per milliliter levels of IL-7 (Fig. 4A). In contrast, at picogram per milliliter IL-7 concentrations, the RAG-2−/− Hc tg pro-B cells show a three- to sixfold higher response than RAG-2−/− pro-B cells. The CD43− pre-B cell population generated in the Hc tg bone marrow was also examined and was unresponsive to IL-7 at any of the concentrations tested (Fig. 4A). These data demonstrate that expression of Ig HC in the absence of conventional Ig LCs is sufficient to modulate the IL-7 dose-response threshold at the pro-B cell stage and shut down IL-7 responsiveness at the pre-B cell stage.

The effect of enforced pre-BCR expression on the kinetics of the IL-7 response was examined by measuring proliferation of RAG-2−/− and RAG-2−/− Hc tg pro-B cells on days 3, 4, 5, and 6 of culture in the presence of saturating levels of IL-7 (Fig. 4B). Interestingly, the response of the RAG-2−/− Hc tg pro-B cells peaked earlier than that of the RAG-2−/− pro-B cells, with the highest response on day 3 and a marked decline by day 5. In contrast, proliferation of RAG-2−/− pro-B cells did not reach maximal levels until day 4 of culture, and no significant decrease occurred on days 5 and 6 (Fig. 4B).

Pre-BCR expression limits the duration of IL-7-induced clonal expansion

One hypothesis to explain the effect of enforced pre-BCR expression on the kinetics of the IL-7 response is that expression of pre-BCR leads to differentiation to an IL-7-unresponsive stage during the culture period. This hypothesis would predict that during culture with IL-7, pre-BCR-expressing cells will gradually lose the ability to clonally expand in response to IL-7, while pre-BCR-deficient cells will retain this ability. To test this prediction, we cultured RAG-2−/−, RAG-2−/− Hc tg, or RAG-2−/− Hc tg pro-B cells for 4 days in the presence of high levels of IL-7 and then determined the frequency of cells retaining the ability to clonally expand in IL-7. We found that RAG-2−/− cultures retain a substantially greater frequency of cells clonable in IL-7 than RAG-2−/− or RAG-2−/− Hc tg pro-B cells (1/6 to 1/9 vs 1/27 to 1/40 or 1/48 to 1/90, respectively; Fig. 5A).

Since IL-7 responsiveness is clearly lost during the pro-B to pre-B cell transition in vivo (Figs. 2 and 4), we hypothesized that the pre-BCR-dependent loss of IL-7 responsiveness during in vitro culture could represent an analogous differentiation event. We thus sought to determine whether the IL-7-unresponsive cells arising in normal pro-B cell cultures could be separated from the cells retaining IL-7 responsiveness by virtue of a pre-B vs pro-B cell phenotype. We used CD2 as a marker for pre-B cells (5), since the starting pro-B cell populations are CD2−, and CD2 expression clearly discriminates a pre-B cell population arising in IL-7 cultures of normal, but not pre-BCR-deficient, cells (data not shown). Day 4 cultures were sorted into CD2+/IgM− or CD2+/IgM+ fractions, and it was found that the CD2+ population has completely

lost the ability to form clones in the presence of IL-7, while the CD2− population contains a high frequency of cells clonable in IL-7 (Fig. 5B). These results indicate that culture of normal pro-B cells with IL-7 leads to development of a pre-B cell population that becomes IL-7 unresponsive.

What is the fate of pre-BCR-expressing pro-B cells that differentiate into IL-7-unresponsive pre-B cells during culture with IL-7? To address this issue we conducted experiments designed to follow the fate of individual pro-B cell clones isolated from RAG-2−/−, RAG-2−/− Hc tg, or RAG-2−/− Hc tg bone marrow. Pro-B cells were plated at limiting dilution in the presence of nanogram per milliliter concentrations of IL-7 on the day of purification from bone marrow, and individual clones were identified on day 4 and reassessed on days 7 and 11 of culture. This analysis revealed striking differences between the different genotypes. RAG-2−/−...
of incubation, plates were scored for colony growth as described above. Counted, and limiting dilutions were plated in 96-well plates. After 4 days
dilution (23/96 wells RAG-2
scored for colony growth, and clones arising at the five cell per well di-
plated in limiting dilutions on the day of the sort. On day 4, plates were
m
cells purified from C57BL6 or
2
and data not shown). When the B220
CD43
pre-B cell clones largely survived and continued to increase in size
throughout the 11-day culture period (Fig. 5C). This result is in
accord with data indicating an inability to established long term
proliferating pre-B cell cultures from RAG-2
HCtg bone marrow (5). RAG-2
4 ceased proliferating and died by day 11.

Together these results support the hypothesis that expression of
pre-BCR on pro-B cells ultimately leads to differentiation to a
pre-B cell stage, where proliferation in response to IL-7 is arrested.
In the absence of further survival and/or differentiation signals, this
growth arrest appears to end in cell death.

Loss of IL-7 responsiveness at the pre-B cell stage, but not pre-
BCR-dependent modulation of IL-7 responsiveness at the pro-B
cell stage, is associated with altered expression of IL-7R α-chain

To determine whether the observed pre-BCR-dependent alterations
in IL-7 responsiveness could be accounted for by regulation
of IL-7R expression, we examined surface expression of the IL-7R
α-chain in RAG-2
, RAG-2
, and RAG-2
HCtg bone
2
2
2
mice show
a significantly higher frequency of IL-7R
2
clones, presumably due to
the absence of later developmental stages that are IL-7R
2
(29, 30). When the B220
CD19
CD43
2
pro-B populations are com-
pared, the frequencies of IL-7R
2
expressed in the three geno-
types are more similar, with RAG-2
2
pro-B cells having
44.3–55.3% IL-7R
2
, RAG-2
2
pro-B cells showing 62.9–
65.8%, and RAG-2
2
HCtg cells showing 32.4–48.6% IL-7R
2
cells (Fig. 6A). The levels of IL-7R expressed on the surface of
IL-7R
2
pro-B cells is undistinguishable among the three geno-
types. These results indicate that the differential ability of pre-
BCR-expressing and pre-BCR-deficient pro-B cells to respond to
picogram per milliliter IL-7 concentrations is unlikely to be due to
differential expression of IL-7R α-chain.

Analysis of IL-7R α-chain expression in B-lineage subpopula-
tions in the control RAG-2
2
bone marrow indicates an interesting
pattern of expression during B cell differentiation (Fig. 6B). IL-7R is expressed at the highest levels on CD43
2
pro-B cells, with
CD43
2
pre-B cells showing substantially decreased levels of
expression. Consistent with this result, expression of the HC trans-
gene in RAG-2
2
mice, which restores development of pre-B
cells (5), leads to down-regulation of IL-7R expression in the B-
lineage compartment (Fig. 6A). These findings indicate that the
loss of IL-7 responsiveness at the CD43
2
pre-B cell stage (Figs.
2B and 4A) correlates with reduced expression of IL-7R α-chain.
κ light chain
2
B cells show no detectable expression of IL-7R
(similar to control staining; data not shown), as has been de-
monstrated previously (29–31).

Discussion
Initiation of IL-7 responsiveness is not dependent on the
pre-BCR

The data presented here clearly show that the ability of pro-B cells
to respond in the presence of nanogram per milliliter levels of IL-7
is not dependent on pre-BCR expression. This conclusion is in
apparent conflict with earlier work examining B cell progenitors
from scid mice (33, 34), which concluded that expression of the
HC is required to generate IL-7-responsive cells. Given the results
of our study and others (5) demonstrating IL-7 responses in RAG-
deficient B cell progenitors, it appears that the scid mutation may
interfere with the development of IL-7-responsive pro-B cells for
Cells stained with anti-κ light chain/anti-CD43/anti-IL-7R α-chain on normal and Ig heavy chain−chain/anti-CD43/anti-IL-7R α-chain/anti-B220. B, Cells stained with anti-κ light chain/anti-CD43/anti-IL-7R α-chain/anti-B220. In addition to the gates indicated, cells were gated on the viable lymphocyte population by forward and side scatter. Similar results were obtained in three independent experiments.

reasons other than their lack of pre-BCR expression. Indeed, the present results show that pro-B cells from HC-deficient mice often have greater levels of proliferation than normal pro-B cells in the presence of high levels of IL-7. This increase is probably related to the failure of HC-deficient pro-B cells to differentiate to an IL-7-unresponsive stage (Fig. 5), which leads to a more sustained response.

Pre-BCR-dependent modulation of IL-7 responsiveness during B-lineage differentiation

In contrast to the results at saturating IL-7 concentrations, we find that expression of pre-BCR is required to generate pro-B cells capable of responding to picogram per milliliter levels of IL-7. This result defines a novel and potentially critical regulatory event in early B cell differentiation that is dependent on pre-BCR expression. The ability of pre-BCR to alter the threshold of ligand needed for triggering through IL-7R could bear some analogy to other multireceptor systems in lymphocytes such as Ag receptor-cosignaling systems. For example, the concentration of ligand required for triggering through the BCR can be altered by up to 100-fold when coreceptors such as CD19, CD22, or FcγRIIB1 are coligated (35). In these cases, the modulation of the triggering threshold is thought to occur at least in part through direct alteration of receptor-proximal signaling events by recruitment of regulatory protein tyrosine phosphatases (35, 36).

However, the present data do not distinguish whether pre-BCR signals alter IL-7R signals directly through a relatively simple biochemical cascade (as in the above examples) or indirectly through a more complex cascade of differential events that alter the signaling milieu within the cell. It is possible that the pathway from pre-BCR expression to altered responses through IL-7R involves several intermediate biochemical or transcriptional events or even a discrete differentiation step. In any case, it will be important to identify the regulatory target molecules involved in modulating responses through IL-7R and determine whether responses through other cytokine receptors, particularly the receptors for IL-2, IL-4, IL-9, and IL-15, which share the common subunit γc with IL-7R (37), are modulated in a dose-dependent manner during lymphocyte development.

As IL-7R is thought to mediate both cell survival and proliferation, it will be interesting to determine whether the pre-BCR-dependent modulation of IL-7 responsiveness is due to altered cell survival signals, cell proliferation signals, or both. The present data examining cell recoveries by trypan blue staining (Fig. 3A) show an approximately 50% decrease in viable cell numbers for pre-BCR-deficient pro-B cells after 4 days of culture in picogram per milliliter IL-7 concentrations vs a fourfold increase in viable cells in controls. However, this does not represent compelling evidence for cell death over failure to proliferate, given that these are primary cells that have been run through a FACS sorter before culture. Further studies will be required to address this issue.

We also provide evidence that expression of pre-BCR leads to shortened duration of IL-7-induced clonal expansion by causing differentiation to an IL-7-unresponsive pro-B cell stage. Thus, expression of pre-BCR appears to lead to two sequential alterations of IL-7 responsiveness: 1) the transient modulation of the IL-7 dose response at the pro-B cell stage, followed by 2) the shutdown of IL-7 responsiveness at the pre-B cell stage. These two regulatory events probably represent independent downstream consequences of pre-BCR-dependent signals. Modulation of the dose-response threshold at the pro-B cell stage does not appear to be associated with changes in IL-7R expression and so is likely to be due to altered signaling pathways. In contrast, the shutdown of IL-7 responsiveness at the pre-B cell stage correlates with decreased expression of IL-7R, providing a potential mechanism for this alteration in responsiveness. In this context, it is interesting that our data indicate a possible trend showing a lower frequency of IL-7R− cells in RAG-2−/− HCtg pro-B cells than in RAG-2−/− or RAG-2−/− × pro-B cells (Fig. 6). This apparent trend may be related to the down-regulation of IL-7R expression during the pro-B to pre-B cell transition, in that the early and high level expression of pre-BCR in the heavy chain tg pro-B cells may lead to rapid down-regulation of IL-7R concomitant with CD43 down-regulation, resulting in more frequent inclusion of IL-7R-negative late pro-B cells in the CD43+ pro-B cell gate. Further studies will be required to clearly address this issue.

Previous experiments assessing the effect of a μ transgene on growth factor responses of HC-deficient pro-B cells have given rise to diverging interpretations (5, 15). One study found that expression of a μ transgene greatly decreases proliferative responses when whole bone marrow cells are cultured with IL-7 for 5 days (5). Since the frequency of pro-B cells is reduced in HCtg bone marrow (Ref. 5 and data not shown), and our data suggest that the IL-7-induced proliferative response peaks earlier (day 3) with HCtg pro-B cells, this
study may have underestimated the capacity of these cells to proliferate in response to IL-7. However, the conclusion that pre-BCR expression leads to eventual shutdown of IL-7 responsiveness is in complete accord with our results. Another study concluded that a μ transgene promotes the ability of HC-deficient pro-B cells to undergo contact-independent proliferation in the presence of a stromal cell line in the absence of exogenously added IL-7 (15). Interestingly, it has been shown that in some stromal cell lines, expression of IL-7 mRNA can be dramatically up-regulated by contact with pro-B cells (38). Thus, given the present results, one interpretation of the contact dependence experiment is that the requirement of HC-deficient cells for stromal cell contact simply reflects a requirement for higher levels of IL-7.

Role of IL-7 and pre-BCR in regulating the pro-B to pre-B cell transition

Since expression of pre-BCR appears to regulate IL-7 responsiveness, it could be possible that the role of HC in promoting the pro-B to pre-B transition in vivo is simply to lower the threshold of IL-7 responsiveness in pro-B cells sufficiently to allow survival and continued differentiation in the presence of the limiting amounts of IL-7 present in vivo. However, culture of HC-deficient pro-B cells in the presence of saturating IL-7 levels does not rescue differentiation to the pre-B cell stage, as assessed by surface marker expression and the level of Ig light chain gene rearrangement (39), suggesting that the extended survival and proliferation afforded by superphysiologic IL-7 concentrations are insufficient to overcome the requirement for pre-BCR. In contrast, normal pro-B cells clearly undergo differentiation to the pre-B cell and B cell stages during short term culture with IL-7 (Fig. 3B) (39).

Rolink and colleagues found that long term B cell precursor lines undergo differentiation events when IL-7 is withdrawn from the cultures, suggesting that culture in saturating IL-7 conditions may inhibit differentiation of pro-B cells to pre-B and B cell stages (23). Our present results using short term cultures indicate that this putative inhibitory effect is at best partial, since normal pro-B cells do differentiate into pre-B cells and IgM+ B cells in the presence of ng/ml IL-7 levels, albeit at a lower frequency than observed in the picogram per milliliter cultures (Fig. 3B and data not shown). In light of the data indicating that pre-BCR-deficient pro-B cells fail to proliferate in response to picogram per milliliter concentrations of IL-7 (Figs. 2, 3A, and 4), we consider it most likely that the striking difference in frequency of μ− B cell precursors found in nanogram per milliliter vs picogram per milliliter IL-7 cultures (Fig. 3B) is due to differential proliferation of the μ− cells; however, we cannot rule out the possibility that nanogram per milliliter IL-7 concentrations may also partially inhibit the differentiation of these μ− cells.

Implications for B cell differentiation in the bone marrow

Several lines of evidence indicate that IL-7 may be present in limiting quantities at the sites of B lymphopoiesis in the bone marrow. Many stromal cell lines that support B lymphopoiesis in vitro produce very low levels of IL-7 mRNA and free soluble IL-7 (20, 21). In addition, IL-7 is bound by extracellular matrix components such as fibronectin and heparin sulfate (40, 41), which are produced by stromal cells. IL-7 bound to heparin sulfate was shown to be functionally inactivated (40), suggesting that IL-7 activity may normally be attenuated by binding to the extracellular matrix. There have also been suggestions that the IL-7 made by stromal cells is only released in small quantities and in a specifically regulated manner (42). Lastly, IL-7 tg mice (43, 44) and mice injected with rIL-7 (45) show a large increase in the number of pre-B cells in the bone marrow, suggesting that the amount of pro-B cell proliferation is normally limited by the low availability of IL-7.

If IL-7 is indeed present at limiting levels, cells able to respond to low IL-7 concentrations could have a large proliferative advantage in vivo. Thus, the ability of pre-BCR to modulate the IL-7 dose-response threshold could be critical in providing pre-BCR+ pro-B cells with a proliferative advantage over pre-BCR− pro-B cells, leading to the preferential accumulation of functionally rearranged HC alleles before light chain rearrangement (10, 31, 46, 47).

Together with data from the literature, the results presented here suggest the following model for the role of pre-BCR in regulating IL-7 responsiveness and differentiation (Fig. 7). IL-7R is expressed soon after commitment to the B lineage; however, the earliest B-lineage-committed progenitors do not respond to even high levels of IL-7 (48). In contrast, heat-stable Ag+ early pro-B cells undergoing DIα rearrangement develop the ability to proliferate in response to superphysiologic levels of IL-7. However, our data suggest that before pre-BCR expression, the array of signals transmitted through the IL-7R is insufficient to trigger proliferation at the presumably low concentrations of IL-7 present in the developmental microenvironment. While these cells may not proliferate in response to IL-7 in vivo, it is plausible that IL-7R-mediated signals could play some role in mediating the survival and differentiation of early pro-B cells, as has been previously suggested (28, 49).

Once a productive HC rearrangement is made, pre-BCR is expressed on the cell surface and activates intracellular signaling pathways through associated signaling molecules such as Igα and Ig-β (17, 50, 51). We postulate that this signal directly or indirectly alters the milieu of signaling mediators such that the threshold for IL-7 responsiveness is lowered, allowing the cells to proliferate in response to the low levels of IL-7 present in the microenvironment. After several rounds of division, this proliferative response terminates in differentiation to the pre-B stage and loss of the ability to proliferate in response to IL-7 (2, 25, 52) (Fig. 5). Our data (Fig. 6) as well as those from other studies (53) indicate that this loss of IL-7 responsiveness at the pre-B cell stage correlates with reduced expression of IL-7R.

Thus, through the collaborative action of pre-BCR and IL-7R signals, a pre-B cell pool enriched for cells expressing functional HC proteins would be generated before activation of LC rearrangement.
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