STEPWISE PROGRESSION OF B LINEAGE DIFFERENTIATION SUPPORTED BY INTERLEUKIN 7 AND OTHER STROMAL CELL MOLECULES

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In the bone marrow of postnatal mice, a large number of B lymphocytes are produced daily by continuous differentiation from pluripotent hemopoietic stem cells (1). The major event in this process is the expression of the complete IgM molecules on their surface, which function as the specific receptor to antigen. Because the V regions of Ig are encoded by DNA segments widely dispersed in the germ line genome, each of the segments has to be assembled to form a functional V region gene during early B lineage differentiation (2, 3). By the advent of a method to produce B lineage cell lines by Abelson-murine leukemia virus (A-MuLV)[1] infection (4, 5), the events that occur sequentially along the B cell differentiation pathway have been clarified to a considerable extent (6–9). The most important features learned from these studies are: (*a*) that the process of Ig gene assembly per se is error prone, consequently generating nonfunctional B cells bearing nonproductively rearranged V region genes in both chromosomes (10); and (*b*) that Ig gene assembly proceeds in an orderly sequence starting from the assembly of H chain V gene segments, the first D to J and subsequent V to DJ joinings, and followed by V to J joining of L chain gene segments (6–9).

Despite such a clear picture presented on the process of Ig gene rearrangement, little is understood as to how the cell proliferation during this stepwise and error-prone process is regulated, except the contribution of bone marrow stromal cells (11–15). Recently, two powerful tools capable of dissecting this process have been developed. One is rIL-7 (16, 17) and the other is IL-7-defective stromal cell clone PA6 (18–20). These two tools enable us to divide the signals expressed in the B lymphopoi-

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[1] *Abbreviations used in this paper:* A-MuLV, Abelson murine leukemia virus; *scid*, severe combined immuno deficiency.

esis-supporting stromal cell clone into IL-7 and other yet unidentified molecule(s) expressed in PA6, and to provide them separately. In addition, to investigate the correlation between the growth signal requirement and the Ig gene configuration of B lineage cells, mice with severe combined immune deficiency (*scid*), which was discovered by Bosma et al. (21), were used to provide a good system. Recent studies unequivocally indicated that, although the Ig gene rearrangement occurs, almost all the rearrangements are aberrant in this mouse (22–25), thereby arresting the B cell differentiation at the early step before the expression of the productive H chain gene. Hence, it would be reasonable to expect that the study on the growth signal requirement of B lineage cells in *scid* mice will reveal how the inability of B cells to express a functional H chain gene would affect their growth property.

In the present study, by using a combination of IL-7 and IL-7-defective stromal cell line PA6, we attempted to determine the growth signal requirement of B lineage cells in *scid* mice. Our result demonstrates that B lineage differentiation in *scid* mice is arrested at the point from the precursors (whose growth requires both PA6 and IL-7 into the next stage) where cells are able to proliferate in response to IL-7 alone. Considering that *scid* mutation primarily affects the process of Ig gene rearrangement, this result suggests that the change of the growth signal requirement during B cell development is directed by the expression of functional Ig gene products.

Materials and Methods

Mice. BALB/c and C57BL/6 mice were purchased from Shizuoka Laboratory Animal Center (Shizuoka, Japan). CB17-*scid*/*scid* (*scid*) mice were kindly provided by Dr. S. Habu, Tokai University, Kanagawa, Japan.

Stromal Cell Lines. The stromal cell clones, PA6 (18) and ST2 (15) were used in this study. The hemopoiesis-supporting and IL-7-producing abilities of these cell lines were described previously (20).

Cell Culture. The culture medium for Whitlock-Witte-type long-term culture was used for the stromal cell-dependent bone marrow cell cultures (11, 19). Preparation of bone marrow cells, culture of bone marrow cells on the stromal cell layer, and protocol for medium exchange were as described previously (18–20). The colony-forming cell assay in response to IL-7 (CFU-IL-7; 26) was carried out in α medium (Gibco Laboratories, Grand Island, NY) containing 1.2% methylcellulose (Muromachi Chemical Co., Tokyo, Japan), 30% FCS (HyClone Laboratories, Logan, UT), 1% deionized BSA (Sigma Chemical Co., St. Louis, MO), 0.1 mM 2-ME (Sigma Chemical Co.), and murine IL-7 (10 U/ml). On day 7 of culture, aggregates consisting of >40 cells were scored as a colony.

IL-7. IL-7 was prepared by transfecting IL-7 cDNA clone pSRα IL-7 into COS1 cells, and the supernatant harvested 3 d after the transfection was used (20). Activity of IL-7 was titrated by using IL-7-dependent B cell clone DW34, established in our laboratory (19). The dilution giving 50% of the maximal [^{3}H]thymidine incorporation of DW34 is defined as 1 U.

Limiting Dilution Assay on the PA6 Layer in the Presence of IL-7. The frequency of clonogenic B cells that proliferate on the PA6 layer in the presence of IL-7 was determined by limiting dilution assay. 4 d before the assay, PA6 monolayer was allowed to form in a 96-well cluster dish (3072; Falcon Labware, Oxnard, CA). Before seeding the bone marrow cells, the medium was removed from the well, and varying numbers of cells diluted in 200 μl of the medium containing 20 U/ml of IL-7 were added. Medium exchange was carried out every 4 d by aspirating half of the spent medium and adding 100 μl of fresh medium containing IL-7. 10–12 d after the initiation of culture, total cells in the well were individually harvested by vigorous pipetting, suspended in 200 μl medium, and 50 μl of it was subjected to the light scatter profile analysis by Epics Profile (Coulter Electronics Inc., Hialeah, FL). The well containing >250 cells distributing in the lymphocyte area (approximately twofold more than the

highest cell number that was generated in the IL-7$^-$ wells receiving 1,000 bone marrow cells) was judged as B lineage cell positive. Each cell dilution consisted of 96 wells. The rationale of our assay to determine the presence of B lineage cells was described in Results.

Cell Staining. mAbs against IgM allotypes MB86 (anti-IgM^b; 27) and DS.1 (anti-IgM^a; 28) were conjugated to FITC and used for detecting surface IgM-bearing cells. B220$^+$ cells were detected by staining with the culture supernatant of RA3-6B2 (29) and subsequent FITC-conjugated anti-rat κ mAb (Immunotech, Marseille, France). Stained cells were analyzed by Epics Profile.

Results

Presence of Two Distinct Subpopulations of B Cell Precursors. It is now well established that there exists a pre-B cell that proliferates in response to IL-7 alone (16, 26, 30). This stage of pre-B cells, therefore, can be enumerated by colony assay in the semi-solid medium as CFU-IL-7 (26, 30). On the other hand, we recently identified another distinct subpopulation of B cells whose growth requires both IL-7 and other stromal cell molecule(s) expressed in PA6 (PA6 + IL-7-dependent precursor). This conclusion is based on our previous observation that bone marrow cells precultured on the PA6 layer in the absence of IL-7 could not proliferate in response to IL-7 alone, while the same cell population did proliferate when both PA6 and IL-7 were present in the culture (20). However, this experiment is not sufficient to conclude that the signal that collaborates with IL-7 for the proliferation of PA6 + IL-7-dependent precursor is actually derived from PA6 itself, because various kinds of cells other than B lineage cells or PA6 were present in the culture. To demonstrate the direct involvement of PA6-derived signals in the proliferation of a PA6 + IL-7-dependent precursor, we first attempted to isolate B cell clones by which we are able to investigate the interaction between stromal cell and B lineage cell in the pure form. Several B cell lines were established by culturing the normal bone marrow cells on the B lymphopoiesis-supporting stromal cell clone ST2. The established cell lines were cloned several times on the ST2 layer by seeding at 0.3 cells/well (19, and M. Ogawa, and S. I. Nishikawa, manuscript in preparation). Among these clones, some proliferated only when both PA6 and IL-7 were present in the culture. A result of a typical example, B220$^+$ cμ^- cell clone, DWb6, was presented in Fig. 1. Since no cellular components, except cloned stromal cell line PA6 and cloned pre-B cell line DWb6, were present in this assay culture, PA6 was actually the source of the signal that collaborated with IL-7 to induce the proliferation of DWb6. Furthermore, this result also indicates that the PA6 + IL-7-dependent precursor exists as a subpopulation of B lineage cells distinct from CFU-IL-7.

Clonogenic Assay for PA6 + IL-7-dependent Precursor. The result in the preceding section proved the presence of a PA6 + IL-7-dependent precursor that is distinct from CFU-IL-7. Obviously, this precursor can be enumerated by the limiting dilution assay in the culture containing both PA6 and IL-7. Importantly, this limiting dilution assay also detects CFU-IL-7 together with a PA6 + IL-7-dependent precursor. Moreover, as expected from our previous studies (18–20), myeloid lineage precursors also proliferate on the PA6 layer, which might be misjudged as B cell precursors. Previous papers by Muller-Sieburg et al. (31) or Whitlock et al. (12) using a similar limiting dilution assay suggested that the presence of small round lymphoid cells observed under a phase-contrast microscope was a good indicator

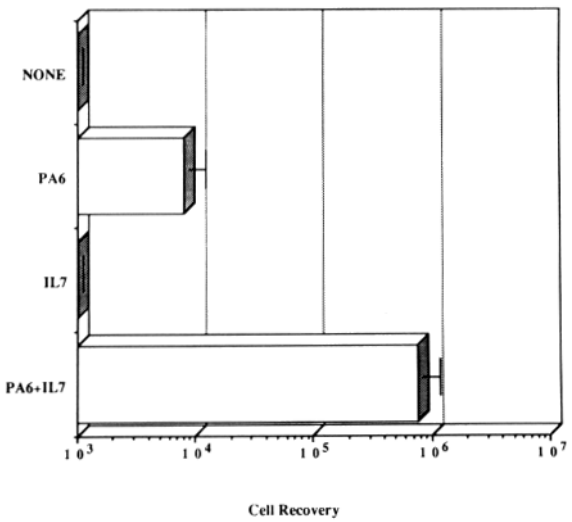

FIGURE 1. Growth signal requirement of stromal cell-dependent B lineage cell clone DWb6. 5×10^4 DWb6 cells were cultured in the presence or absence of either IL-7, the PA6 monolayer, or both. 4 d after the culture, all the cells in the well were harvested, and viable small cells excluding trypan blue were counted. Each column represents the arithmetic mean of triplicate wells.

for the presence of B lineage cells. By a series of our preliminary experiments testing the correlation of this criteria with the actual presence of lymphoid cells identified by May-Grunwald-Giemsa staining, however, we noticed that, at least under our culture condition, considerable numbers of lymphocyte-negative wells were counted as positive. Nevertheless, it is practically impossible to perform cell staining for every assay, each of which consists of 600–1,000 wells. Fortunately, small lymphocytes are easily discriminated from other hemopoietic cell lineages by their light scatter profile. As shown in Fig. 2, in the bone marrow cells from *scid* mice, there exist only a few cells in the lymphocyte area (the small circle in the figure) that were set to cover the majority of normal spleen cells. In contrast, in normal bone marrow cells, a considerable number of cells were distributed in this area. Therefore, light scatter analysis is a feasible way to screen many wells at the same time. Furthermore, this method allows us to measure the exact cell recovery by directly counting the cell number in each well.

To further test the validity of this criteria, we next measured the frequency B cell precursor clonable on the PA6 layer under B lymphopoietic or non-B lymphopoietic

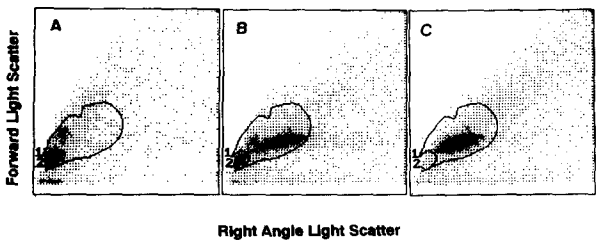

FIGURE 2. Light scatter profile of various cell populations. Spleen cells from a BALB/c mouse (*A*), bone marrow cells from a BALB/c mouse (*B*), and bone marrow cells from a *scid* mouse (*C*) were analyzed for their light scatter profile by Epics Profile. The large circle (*1*) was set so as to cover a majority of bone marrow cells. The small circle (*2*) covered a majority of spleen cells from the BALB/c mouse, and was used as the lymphocyte area throughout this study.

conditions, namely in the presence or absence of IL-7. Varying numbers of normal bone marrow cells were cultured on the PA6 layer in the presence or absence of IL-7. After 12 d, the cells were harvested and analyzed for their light scatter profile, and the number of the cells distributed in the lymphocyte area was counted by Epics Profile. As shown in Fig. 3, no positive well was able to be scored when the limiting dilution assay was performed in the absence of IL-7. On the other hand, if IL-7 was present in the culture, we obtained the straight line through the origin when the numbers of cells seeded were plotted in a semilogarithmic plot. This result indicates that IL-7 is absolutely required for measuring the frequency of PA6 + IL-7-dependent cells, and our method is specific to the B progenitors. In all the IL-7$^-$ wells receiving 1,000 bone marrow cells, proliferation of the cells was clearly observed. A typical example of these wells was presented in Fig. 4, showing that virtually no small lymphocytes or B220$^+$ cells were present in the culture. Although varied amounts of background counts in the lymphocyte area were always detected in these wells, the highest number obtained among the 96 wells of this group was 128, and the arithmetic mean and SD was 67 ± 38. On the other hand, among the IL-7$^+$

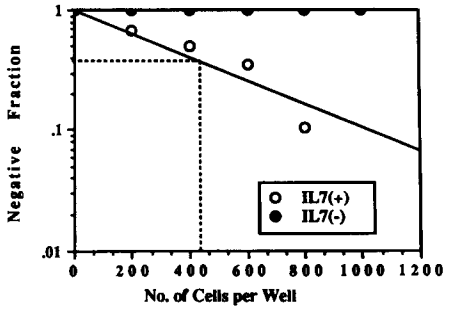

FIGURE 3. Limiting dilution analysis of clonogenic B precursor on the PA6 layer in the presence or absence of IL-7. Bone marrow cells were harvested from a BALB/c mouse, and 200, 400, 600, 800, and 1,000 cells were inoculated in a well of a 96-well cluster dish containing the PA6 monolayer. The cells were cultured in the presence or absence of IL-7 for 12 d, and the cells in each well were suspended in 200 μl of medium. 50 μl of aliquot was analyzed by Epics Profile, and the number of cells in the lymphocyte area was counted. If the total number of small lymphocytes in a well exceeded 250 cells, the well was judged as positive for the clonogenic B precursor. Each cell dilution consisted of 96 wells.

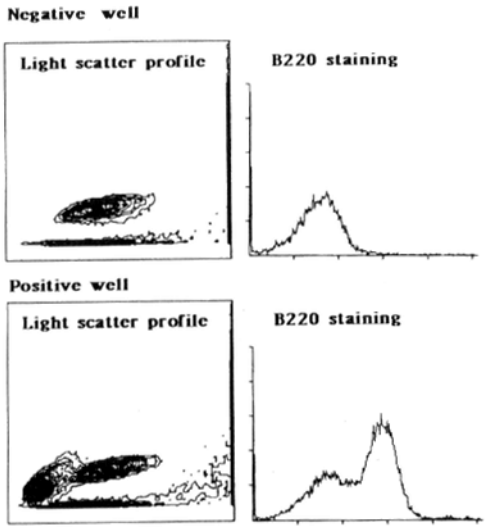

FIGURE 4. Light scatter profile and B220 staining of the cells obtained from negative and positive wells in the limiting dilution assay. Some of the wells presented in the plot of Fig. 3 were further analyzed for the presence of B220$^+$ cells. Two examples were presented: one is from an IL-7$^-$ well receiving 1,000 bone marrow cells, and the other is from a IL-7$^+$ wells receiving 200 bone marrow cells. Right panels represented the anti-B220 staining pattern of all the cells distributed in the area that covered both lymphoid and myeloid cells (large circle [*1*] in Fig. 2).

wells receiving only 200 of the same bone marrow cells, 31 of 96 wells were positive with our criteria (among 31 positive wells, four contained 250–500 small lymphocytes, 15 contained 500–2,500 small lymphocytes, eight contained 2,500–12,500 small lymphocytes, and four contained >12,500 small lymphocytes), and the cells in the lymphocyte area included $B220^+$ cells (Fig. 4). Furthermore, varying numbers of $sIgM^+$ cells (0.8–28%) were also detected in all of the positive wells that generated >2,000 cells in the lymphocyte gate. This result indicates that our assay basically detects any B precursors that proliferate and give rise to >250 small lymphocytes in the lymphocyte area on the PA6 layer in the presence of IL-7.

Frequency of Clonogenic B Cells in the Bone Marrow Cells of Normal and scid Mice. Using a combination of the limiting dilution assay described in the preceding section and the CFU-IL-7 assay, frequencies of CFU-IL-7 and PA6 + IL-7-dependent precursor were enumerated. Fig. 5 represents the plot of limiting dilution assay for a PA6 + IL-7-dependent precursor in the bone marrow of BALB/c or *scid* mice. We could detect a PA6 + IL-7-dependent precursor in the bone marrow of *scid* mice as well as that of BALB/c mice, although the frequency was markedly lower in the *scid* bone marrow. To confirm that the cells generated in the culture of *scid* mice were actual B lineage cells, all the positive wells that contained >2,000 cells in the lymphocyte area were further subjected to the surface staining analysis with anti-B220 antibody (16 examples were shown in Fig. 6). In all the lymphocyte-positive wells tested, $B220^+$ cells were present. This indicates that PA6 + IL-7-dependent precursors in the bone marrow of *scid* mice are indeed B cell precursors. The frequencies calculated from Fig. 5 were presented together with those of CFU-IL-7 in Table I. This result confirmed the previous observation of ours and another group that CFU-IL-7 was absent in the bone marrow of *scid* mice (26, 30). In addition, the presence of a PA6 + IL-7-dependent precursor was clearly shown in *scid* bone marrow.

PA6 + IL-7-dependent Cell Is the Direct Precursor of CFU-IL-7, and the Differentiation of PA6 + IL-7 Precursor into CFU-IL-7 Is Arrested in scid Mice. The result in the preceding section can be interpreted to suggest either that CFU-IL-7 and PA6 + IL-7-dependent precursor belong to two different lineages of B cell differentiation

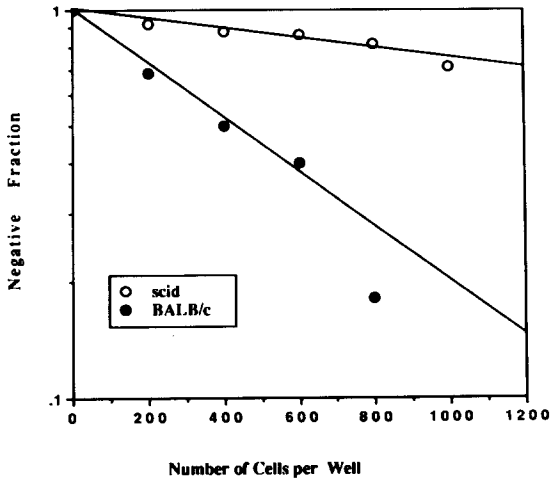

FIGURE 5. Limiting dilution assay for PA6 + IL-7-dependent precursors in BALB/c and *scid* bone marrow. Bone marrow cells were harvested from either a BALB/c or *scid* mouse, and 200, 400, 600, 800, and 1,000 cells were cultured in a well of a 96-well cluster dish containing the PA6 monolayer and IL-7. 11 d after the culture, the cells in each well were subjected to the light scatter analysis. Each cell dilution consisted of 96 wells. The frequency calculated from this plot was presented in Table I together with the result of the CFU-IL-7 assay on the same preparations of bone marrow cells.

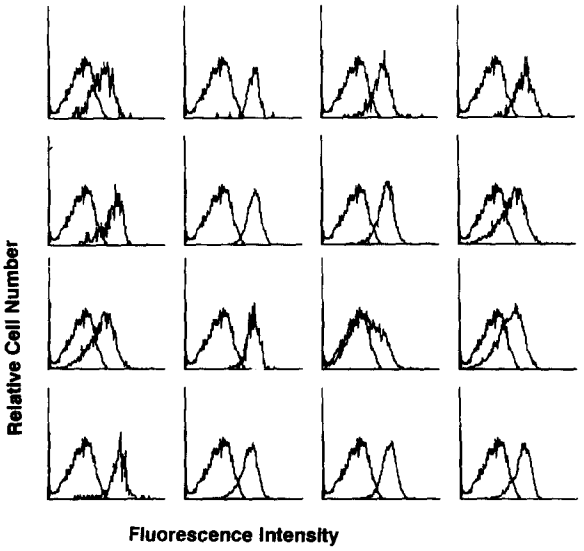

FIGURE 6. Presence of B220$^+$ cells in the small lymphocytes generated in the culture of *scid* bone marrow. The cells in the small lymphocyte-positive wells of the plot presented in Fig. 5 were further stained with anti-B220 antibody. 16 examples from the culture of *scid* bone marrow cells were presented together with control staining. The results represent the staining pattern of the cells within the area covering only small lymphocytes (small circle [*2*] in Fig. 2).

and the former is lost in *scid* mouse, or that these two are allocated on the same differentiation pathway and the differentiation from PA6 + IL-7-dependent precursor into CFU-IL-7 is arrested in *scid* mice. If the cell population containing PA6 + IL-7-dependent precursor but no CFU-IL-7 is available and CFU-IL-7 is generated from this population, we can conclude that the latter is the actual case. In a previous paper, we demonstrated that when bone marrow cells were cultured on the PA6 layer in the absence of IL-7, the cells that can proliferate in response to IL-7 alone were lost, while PA6 + IL-7-dependent precursors were preserved (20). Hence, in this work, we started from this cell population and investigated whether or not CFU-IL-7 is generated.

The bone marrow cells from C57BL/6 or *scid* mice were cultured on the PA6 layer in the absence of IL-7. 2 wk later, the cells were harvested and transferred onto the new culture that contained both PA6 and IL-7. Before and 2 wk after the transfer, the frequencies of CFU-IL-7 and PA6 + IL-7-dependent precursor were measured (Fig. 7). Confirming our previous result, CFU-IL-7 was lost when bone marrow cells were cultured on the PA6 layer in the absence of IL-7. In contrast, PA6 + IL-

TABLE I

Frequencies of PA6 + IL-7-dependent Precursor and CFU-IL-7 in the Bone Marrow of BALB/c and scid Mice

Mouse	BALB/c	*scid*
PA6 + IL-7-dependent precursors	1/533	1/3,028
CFU-IL-7	1/1,111	$<1/10^5$

Bone marrow cells were harvested either from a BALB/c or *scid* mouse. The frequency of the PA6 + IL-7-dependent precursor was calculated from the plot in Fig. 4. A CFU-IL-7 assay was also performed on the same cell populations.

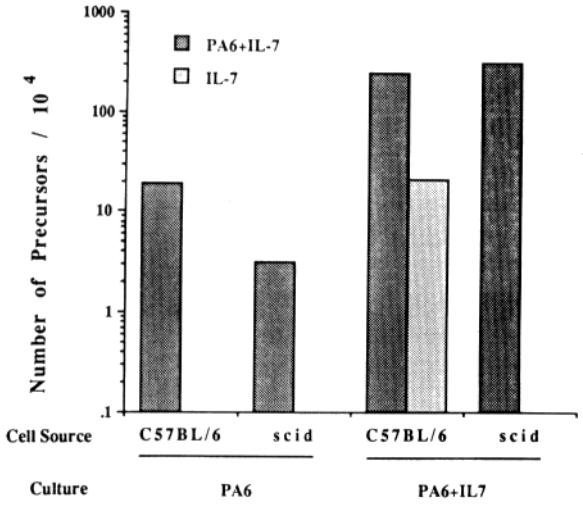

FIGURE 7. Blockade of the differentiation from PA6 + IL-7-dependent precursor into CFU-IL-7 in *scid* mouse. 2×10^6 bone marrow cells from C57BL/6 or *scid* mouse were cultured in a 25-cm^2 flask containing the PA6 layer but not IL-7. 2 wk later, the cells were harvested and transferred onto the secondary culture in the flask containing both the PA6 layer and IL-7. Before and 2 wk after the secondary culture, the frequencies of PA6 + IL-7-dependent precursor and CFU-IL-7 were measured.

7-dependent precursor was present in the cultures of both C57BL/6 and *scid* mice. Importantly, its frequency was again fivefold less in *scid* culture than C57BL/6 culture, suggesting that the lower frequency of PA6 + IL-7-dependent precursor in *scid* bone marrow is not due to the loss of CFU-IL-7 that is included in this frequency. On the other hand, when the bone marrow cells of normal mice precultured on the PA6 layer were transferred onto the new culture containing both PA6 and IL-7, CFU-IL-7 was generated. This clearly demonstrates that CFU-IL-7 is differentiated directly from PA6 + IL-7-dependent precursor along with its proliferation. Interestingly, the low frequency of PA6 + IL-7-dependent precursor in the *scid* culture was corrected after addition of IL-7, and in this particular experiment the frequency was higher than that of normal mice. Despite such an active proliferation of PA6 + IL-7-dependent precursor, however, no CFU-IL-7 was generated in the culture of *scid* mice. This result suggests that the differentiation from PA6 + IL-7-dependent precursor into CFU-IL-7 is completely arrested in *scid* mice.

Discussion

Two Distinct Stages during B Cell Differentiation. Although it is well established that intramarrow B lymphopoiesis is regulated by the stromal cell components (1, 11–15), it has not been clear how the stromal cell regulates this process. The cloning of cDNA coding for IL-7 (16, 17), however, opened a new opportunity to study the molecular mechanisms in this process. Several groups showed that a considerable fraction of early B cell precursor was stimulated by IL-7 to proliferate, suggesting the important role of IL-7 in intramarrow B lymphopoiesis. In a recent review of Henny (32), it was proposed that IL-7 is a growth factor acting on the B precursors from earliest $B220^-$ pro-B to $c\mu^+$ pre-B cells, and the role of other stromal cell molecules is to regulate the differentiation. On the other hand, Lee et al. (30) and Suda et al. (26) demonstrated that the target cells of IL-7 are $B220^+$ pre-B cells. Moreover, the latter showed that $B220^+$ $sIgM^-$ cells purified by FACS were stimulated by IL-7 to form a colony that contained $sIgM^+$ cells (26), suggesting that spontaneous maturation

of B cells occurs during the pre-B cell proliferation. Since this observation forms a clear contrast to the conclusion of Lee et al. (30), it still remains to be investigated whether spontaneous B cell maturation occurs during expansion of the pre-B cells or maturation is arrested during proliferation of pre-B cells in response to IL-7.

Apart from this controversy concerning the late stage of B cell differentiation, we have been proposing that there should be another stage of B lineage cells that require both PA6 and IL-7 for their proliferation (20). The clearest evidence for the presence of this stage is that there exist cloned cell lines whose growth requires both IL-7 and PA6 (Fig. 1). This cell line has already been described as the cell line that proliferates on the ST2 layer but not on the PA6 layer (19). The present study further extended this finding and showed that IL-7 is indeed the additional factor required together with PA6 for its proliferation. Since this cell line has been cloned several times on the ST2 layer and subsequently on the PA6 layer, possible contamination of other hemopoietic cell lineages or fresh bone marrow stromal cells can be ruled out. Therefore, the additional signal(s) to IL-7 required for the proliferation of DWb6 must be derived from PA6 itself. The second evidence for PA6 + IL-7-dependent precursor is that B precursors in the bone marrow of *scid* mouse proliferated only when both PA6 and IL-7 were provided in culture (Fig. 4). Third, CFU-IL-7 were lost during the culture of bone marrow cells on the PA6 layer in the absence of IL-7, while PA6 + IL-7-dependent precursor cells were maintained there (Fig. 6). Finally, our recent study showed that PA6 + IL-7-dependent precursors were detected in the fetal liver at day 10 of gestation, whereas first CFU-IL-7 appears 3–4 d later (M. Ogawa and T. Suda, unpublished observations). These lines of evidence suggest unequivocally that there exists the distinct stage of B lineage cells whose proliferation requires both PA6 and IL-7. At present, the role of PA6 for the proliferation of PA6 + IL-7-dependent precursor remains to be elucidated, though it seems likely that PA6 is required for the continuous expression of IL-7-R. In this context, it is of interest to note that the recent study of Watson et al. (33) shows that an additional signal from the thymic stromal cell works synergistically with IL-7 for inducing the proliferation of fetal thymocytes. Thus, it could be that the IL-7 + stromal cell-dependent stage is the obligatory step for both T and B lymphocytes to acquire the ability to proliferate in response to IL-7 alone.

In this study we demonstrated that addition of IL-7 into the culture of bone marrow cells on the PA6 layer resulted in the induction of CFU-IL-7. Because the initial cell population contained PA6 + IL-7-dependent precursors but no CFU-IL-7, it is very likely that CFU-IL-7 is differentiated directly from a PA6 + IL-7-dependent precursor. It is unlikely, however, that IL-7 itself induces the differentiation of PA6 + IL-7-dependent precursor into CFU-IL-7, because the same cell population did not produce CFU-IL-7 when cultured with IL-7 alone (data not shown). Thus, the presence of both PA6 and IL-7, which allows the expansion of PA6 + IL-7-dependent precursor, is the absolute requirement for the generation of CFU-IL-7 from the bone marrow culture on the PA6 layer. Hence, we prefer to think either that PA6 is the source of the differentiation signal, or that, once the differentiation is triggered, B cell maturation proceeds autonomously if proliferation of the cells is supported by external growth factors. In conclusion, based on the present study and a previous study on the surface character of CFU-IL-7 (26), we propose the following hypothesis on the change of the growth signal requirement during B

cell development. PA6 + IL-7-dependent precursor is generated from the earlier precursor on the PA6 layer in the absence of IL-7. When IL-7 is available together with other stromal cell molecules, they proliferate extensively and some, but not all, of them differentiate into the CFU-IL-7 stage. Finally, upon maturation of pre-B cell into $sIgM^+$ B cell, IL-7 reactivity was switched off and its proliferation requires other signals than those derived from bone marrow stromal cells. Obviously our model should be critically tested, particularly in relevance to the model described by Henny (32), which came out of the investigation of Namen and colleagues (16, 17, 30). For this purpose, IL-7-defective stromal cell clone PA6 together with rIL-7 will be useful.

The Defect of scid Mice Implies the Mode of B Cell Differentiation. Recent studies on the defect of *scid* mice suggested that *scid* mutation affects primarily the process of gene assembly of Ig and TCR gene segments (21–25). More recently, Hendrickson et al. (25) and Lieber et al. (34) introduced the plasmid containing recombination signal sequences for Ig gene recombinase into the B cell lines from *scid* mice and demonstrated that the majority of the rearranged form of the plasmids recovered from these cell lines were aberrantly rearranged. Finally, Scott and colleagues (35) demonstrated that the absence of thymocytes in *scid* mice was cured by the introduction of functional α and β TCR genes, although T cell repertoire in this transgenic *scid* mouse was restricted. All these indicate unequivocally that no other process than antigen receptor gene rearrangement is disturbed directly by *scid* mutation. Nevertheless, our present results, as well as previous studies (26, 30), showed clearly that CFU-IL-7 was absent in *scid* bone marrow. The present result further extended this observation and demonstrated that the B lineage differentiation in *scid* mice is arrested at a point somewhere between the PA6 + IL-7-dependent stage and CFU-IL-7. This result may explain the reason for the previous observation that long-term B cell culture can be established from bone marrow cells from *scid* mice (36, 37). Nevertheless, why can *scid* mutation that primarily affects the Ig gene rearrangements disturb the B cell differentiation from PA6 + IL-7-dependent stage to CFU-IL-7? One plausible explanation for this may be that expression of functional H chain is the requisite step to trigger the shift of growth signal requirement from PA6 + IL-7-dependent precursor to CFU-IL-7. If this is the case, during the B cell development in *scid* mice where the frequency of productive H chain rearrangement is extremely low, PA6 + IL-7-dependent precursors can not differentiate into CFU-IL-7. Of interest to note in this context is the previous observation of Lee et al. (30) that ~80% of cells proliferating in response to IL-7 alone were $c\mu^+$ (30), suggesting that the majority of CFU-IL-7 expressed a functional H chain. Interestingly, expression of functional L chain that occurs after the H chain gene rearrangement also seems to be an important signal involved in the process that switches off the IL-7 reactivity. This was best indicated by the results of Lee et al. (30) and Suda et al. (26) that IL-7 reactivity was lost in the $sIgM^+$ cells. Taken collectively, it seems that change of growth signal requirement during B cell development may be directed by the result of Ig gene rearrangement, and only those that undergo productive Ig gene rearrangement at each step are allowed to enter the subsequent step with new growth properties, whereas those that failed in this process have to stay at the same stage.

One question that remains to be elucidated is the reason for the reduction of the frequency of PA6 + IL-7-dependent precursor in the bone marrow of *scid* mice.

Since the same magnitude of reduction in the frequency was still apparent after culturing the bone marrow cells on the PA6 layer, this phenomenon would be the result of the earliest series of events occurring on the PA6 layer. To further dissect this earliest process, identification of the PA6-derived molecules controlling the differentiation of pluripotent stem cells into B cell lineages is awaited.

Summary

Growth of early B precursor cells was investigated in vitro by using rIL-7 and IL-7-defective stromal cell line PA6 as separate growth signals. B cell development proceeds through three sequential stages different from the growth signal requirement. The cells in the first stage require PA6 alone for the proliferation, and differentiate into the second stage, which requires both PA6 and IL-7 for its growth. When IL-7 is available for the cells in the second stage, they proliferate extensively on the PA6 layer, and some acquire the ability to proliferate in response to IL-7 alone. This sequential change of growth signal requirement, however, does not proceed autonomously along the time schedule. The possibility that it is primarily directed by the result of Ig gene rearrangement is considered. This mode of growth control may explain why only functional B cells are selected in the error-prone process of Ig gene rearrangement during B lineage differentiation.

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