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*J Immunol* 1998; 161:4611-4617; [http://www.jimmunol.org/content/161/9/4611](http://www.jimmunol.org/content/161/9/4611)

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IL-7 Reconstitutes Multiple Aspects of v-Abl-Mediated Signaling

Arnob Banerjee* and Paul Rothman†

The mechanism by which early lymphoid cells are selectively transformed by v-Abl is currently unknown. Previous studies have shown constitutive activation of IL-4 and IL-7 signaling pathways, as measured by activation of Janus protein kinase (JAK)1, JAK3, STAT5, and STAT6, in pre-B cells transformed by v-Abl. To determine whether activation of these cytokine signaling pathways by v-Abl is important in the cellular events induced by the Abelson murine leukemia virus, the effects of IL-4 and IL-7 on pre-B cells transformed with a temperature-sensitive v-Abl mutant were examined. Whereas IL-4 had little or no effect, IL-7 delayed both the apoptosis and cell cycle arrest that occur upon v-Abl kinase inactivation. IL-7 also delayed the decreases in the levels of c-Myc, Bcl-2, and Bcl-xL that occur upon loss of v-Abl kinase activity. IL-7 did not maintain v-Abl-mediated differentiation arrest of the pre-B cells, as activation of NF-κB and RAG gene transcription was unaffected by IL-7. These results identify a potential role for IL-7 signaling pathways in transformation by v-Abl while demonstrating that a combination of IL-4 and IL-7 signaling cannot substitute for an active v-Abl kinase in transformed pre-B cells. The Journal of Immunology, 1998, 161: 4611–4617.

Activated forms of the c-Abl nonreceptor tyrosine kinase, such as v-Abl and BCR-Abl, have been shown to specifically transform hemopoietic cells. The principal targets of the Abelson murine leukemia virus (A-MuLV), which encodes the v-Abl tyrosine kinase, are lymphoid precursors (1). Characterization of the function of v-Abl in pre-B cells has used a virus encoding a temperature-sensitive mutant of v-Abl (ts-A-MuLV); this virus possesses kinase (transforming) activity at a permissive temperature (34°C) but is kinase inactive at a nonpermissive temperature (39°C). Studies using ts-A-MuLV-transformed cell lines revealed that upon shift to a nonpermissive temperature, the pre-B cells begin to undergo cell cycle arrest and apoptosis (2, 3). It is unclear which signaling pathway or combination of pathways support survival and proliferation in these cells, as v-Abl activates multiple signaling molecules, including phosphatidylinositol 3-kinase, c-Myc, protein kinase C, c-Fes, and Ras/mitogen-activated protein kinase (4–8). Interestingly, differentiation arrest of the transformed cells at the pre-B stage is also lost at a nonpermissive temperature. Evidence of differentiation in previous studies included increases in the transcription of RAG-1 and RAG-2 genes and the detection of germline κ transcripts along with rearrangement of the Igκ locus (9). These studies suggest that the regulation of apoptosis, cell cycle progression, and/or differentiation arrest by v-Abl may be required for transformation by A-MuLV.

Recent work from our laboratory has demonstrated that Janus protein kinases (JAKs) and STATs, specifically JAK1, JAK3, STAT5, and STAT6 are activated constitutively in A-MuLV-transformed pre-B cell lines (10). Activation of these molecules in pre-B cells is characteristic of IL-4 (STAT6) and IL-7 (STAT5) stimulation. Since then, several groups have observed constitutive STAT activation in BCR-Abl transformed cells (11–13). Activation of JAK-STAT pathways has been studied extensively and found to be a critical element of cytokine signal transduction. The observation that JAKs and STATs are activated by oncogenic forms of c-Abl is interesting because it suggests the involvement of cytokine-stimulated survival and proliferation pathways in the process of lymphoid transformation. Although activation of cytokine signaling molecules in v-Abl- and BCR-Abl-transformed cells has been established, it remains unclear whether these cytokine pathways are important for transformation by Abl and, if so, which Abl-induced effects they mediate.

Although IL-4 has been shown to have antiapoptotic activity and promote proliferation in B cells and T cells (14), it has not yet been implicated in hemopoietic malignancy. In contrast, two studies have found that overexpression of IL-7 may play a role in transformation. In one of these studies, transgenic mice were generated in which IL-7 was expressed under control of the Ig heavy chain promoter and enhancer. These mice had dermal lymphoid infiltrates composed of T lineage cells and also developed B and T cell lymphomas within the first four months of life (15). In the second study, transgenic mice were generated in which IL-7 expression was under the MHC class II promoter. These transgenics had a high incidence of lymphoid tumors by 4 to 6 mo of age (16). In addition to these transgenic studies, the role of autocrine IL-7 signaling in the transformation of pre-B cells has been examined by two groups. One of these studies demonstrated that although expression of IL-7 could promote transformation of pre-B cells, there were other events that were also required for pre-B cell transformation (17). Another study found that although v-Abl transformation of a pre-B cell line sometimes resulted in autocrine IL-7 production, overexpression of IL-7 in the same cell line was not...
sufficient for transformation (18). Thus, these studies suggest that although dysregulated IL-7 signaling is not sufficient for pre-B cell transformation, it may contribute to this process. However, it is not known how constitutive IL-7 signaling contributes to transformation.

The activation of IL-4 and IL-7 signaling molecules in A-MuLV-transformed pre-B cells suggests that perhaps signaling through a combination of these cytokine pathways could replace v-Abl kinase activity in transformed pre-B cells. To examine this possibility, we used a ts-A-MuLV-transformed pre-B cell line to determine whether IL-4 and IL-7 signaling could compensate for the loss of v-Abl kinase activity. These studies reveal that IL-7 but not IL-4 can reduce the apoptosis and cell cycle arrest that result from v-Abl kinase inactivation in these cells. Additionally, both v-Abl and IL-7 were found to regulate levels of c-Myc, Bcl-2, and Bcl-xL. IL-7 does not alter the activation of NF-κB or the induction of RAG-1 and RAG-2 gene expression at a nonpermissive temperature. These findings demonstrate that reconstitution of v-Abl-stimulated JAK-STAT activation in A-MuLV-transformed pre-B cells by IL-4 and IL-7 is not sufficient to maintain the cells upon v-Abl inactivation, although IL-7 can transiently promote cell survival and proliferation.

Materials and Methods

Cell culture and cytokines

The 103 (ts-A-MuLV transformed pre-B cells) and 103/bcl-2-4-2 cells (103 cells expressing human bcl-2) (9) were grown in RPMI 1640 supplemented with 10% heat inactivated FCS and 50 μM B-mercaptoethanol. Cells were grown routinely at 34°C, the permissive temperature for ts-A-MuLV mutants. The nonpermissive temperature used in all experiments was 39°C. Additions of cytokine consisted of 10 U/ml murine rIL-7 (Genzyme, Cambridge, MA), 400 U/ml murine rIL-4 (a generous gift of Dr. Robert Coffman, DNAx, Palo Alto, CA), or both.

Apoptosis assays

For PI staining, cells were washed once with cold PBS and fixed in cold 80% ethanol for 30 min. The samples were resuspended in buffer containing 0.05 mg/ml PI, 0.3% Nonidet P-40, and 1 mg/ml RNase A; incubated for 30 min at room temperature; and analyzed via FACS. Cell viability was also monitored by trypan blue exclusion.

Northern blot analysis

RNA was extracted by the LiCl method. Briefly, harvested cells were homogenized in 3 M LiCl/6 M urea and kept overnight at 4°C. Samples were centrifuged, and the pellets were resuspended in 10 mM Tris (pH 7.6), 1 mM EDTA, 0.5% SDS, and an equal volume of phenol:chloroform (1:1). Samples were centrifuged, and the pellets were resuspended in 10 mM Tris (pH 7.6), 1 mM EDTA, and incubated at 4°C. DNA binding reactions were performed as described previously (19) in 10-μl reaction volumes containing 40 mM KCl, 1 mM MgCl2, 0.1 mM EGTA, 1 mM HEPEs (pH 7.9), 4% Ficoll, 1.2 mg/ml BSA, 200 μg/ml dl-dC, 10 μg of extract, and 1 ng of probe. After a 20-min incubation at room temperature, binding reactions were fractionated on 0.22× Tris-buff- ered EDTA/4.5% acrylamide (29/1) gel. The probe used was end-labeled, double-stranded oligonucleotide from the IFN response factor-1 promoter (−138 to −107) TACAACACCTGATTCCCGGAATGCGC.

Results

IL-7 delays apoptosis induced after the loss of v-Abl kinase activity

Experiments with the 103 cell line, a ts-A-MuLV-transformed pre-B cell line, have shown that the cells undergo apoptosis upon loss of v-Abl kinase activity; complete cell death is seen by 4 days (9). To determine whether culture with IL-4, IL-7, or a combination of these two cytokines could prevent apoptosis resulting from v-Abl inactivation, 103 cells cultured with or without cytokines were incubated at a nonpermissive temperature. Cells were harvested after 16, 24, and 36 h, and the number of apoptotic cells was determined by either PI (a marker of DNA content) staining or trypan blue exclusion. PI staining was used to identify apoptotic cells in which DNA degradation had begun, leaving the cells with less than one diploid unit of DNA (where one diploid unit is defined as the amount of DNA in cells in G1 phase of the cell cycle). Cells cultured with IL-7 for 16 h at a nonpermissive temperature had a significant reduction in cells undergoing apoptosis when compared with cells cultured without IL-7 (Fig. 1A). These results were verified by trypan blue exclusion assays (Fig. 1B). In cells cultured with IL-7, the percentage of viable cells was 35.0% and 15.5% at the 24-h and 36-h timepoints, respectively. In contrast, in cells cultured without IL-7, the percentage of viable cells was 18.7% and 3.7% at the 24-h and 36-h timepoints, respectively. Addition of IL-4 to the culture had no effect on cell viability at a nonpermissive temperature. These results indicate that IL-7 decreases the percentage of apoptotic cells in ts-A-MuLV-transformed pre-B cells in which v-Abl has been inactivated and suggest that v-Abl and IL-7 may activate some common survival signals in these cells.

IL-7 and v-Abl have both been linked to the regulation of bcl-2 family members. bcl-2 was the first identified member of a family of proteins that regulate cell survival. Other family members include bax, which promotes apoptosis, and bcl-xL, which has two splice variants, Bcl-xL and Bcl-xS (20). Of these two forms, Bcl-xL, like Bcl-2, has antiapoptotic activity, whereas Bcl-xS has apopptic properties. Previous studies have shown that cells transformed by BCR-Abl express Bcl-2; in addition, such cells revert to growth factor-dependence and lose tumorigenic potential upon suppression of Bcl-2 expression (21). Recently, Bcl-xS up-regulation by v-Abl was demonstrated in a pre-mast cell line (22). To determine whether v-Abl can regulate levels of Bcl-2 in pre-B cells, 103 cells were shifted to a nonpermissive temperature and...
examined for Bcl-2 expression. Northern blot analysis demonstrated that Bcl-2 transcript levels remained unchanged for 2 h after the cells were placed into a nonpermissive temperature but fell to nondetectable levels in cells cultured at 39°C for 6 h (Fig. 2A, lanes 1–3). Western blot analysis of whole cell extracts from these cells demonstrated that Bcl-2 protein levels declined after cells were cultured at nonpermissive temperature for 6 h (Fig. 2B, lanes 1–4). These data show that Bcl-2 levels are regulated by v-Abl in A-MuLV-transformed pre-B cells.

To determine whether the ability of IL-7 to delay apoptosis in 103 cells at a nonpermissive temperature could involve bcl-2 regulation, each culture condition in the above experiments was repeated in the presence of IL-7 (Fig. 2A, lanes 4–5 and Fig. 2B, lanes 5–7). Culturing cells with IL-7 increased the levels of bcl-2 transcripts detected in these cells at the 2-h timepoint. However, bcl-2 transcripts were still not detectable in cells cultured at 39°C for 6 h. Bcl-2 protein levels remained at detectable levels for a longer period at a nonpermissive temperature when cells were treated with IL-7; levels fell off between 6 and 12 h. These data demonstrate that IL-7 can stabilize bcl-2 expression levels in v-Abl-transformed pre-B cells.

To determine whether v-Abl or IL-7 could affect the levels of other bcl-2 family members, levels of Bcl-x and Bax were also examined by Western blot analysis (Fig. 2B). Whereas Bcl-x S expression was not observed in these cells (data not shown), Bcl-x L appeared to be regulated by both v-Abl and IL-7. Bcl-x L protein levels declined after 6 h of culture at a nonpermissive temperature, and this decline was prevented by the addition of IL-7 to the culture. Decreases in Bcl-x L levels appeared to occur later than decreases in Bcl-2, both in the presence and absence of IL-7. The levels of Bax, a proapoptotic member of this family of proteins, did not change either with the loss of v-Abl kinase activity or with the addition of IL-7 to the cell cultures. The above results suggest that the suppression of apoptosis by both v-Abl and IL-7 may be at least in part due to regulation of bcl-2 or bcl-x L.

IL-7 decreases cell cycle arrest induced by v-Abl inactivation

Studies using ts-A-MuLV-transformed pre-B cells demonstrate that these cells arrest in G1 phase of the cell cycle following 21 h...
of v-Abl inactivation (2). It is possible that in addition to decreasing apoptosis, IL-7 could also delay or prevent cell cycle arrest in these cells. To address this possibility, the distribution of cells in the different phases of the cell cycle was examined in ts-A-MuLV-transformed pre-B cells cultured at a nonpermissive temperature in the presence or absence of IL-7. The 103 cells were cultured with or without IL-7 and shifted to a nonpermissive temperature for 6, 12, or 18 h. BrdUrd (a marker of DNA synthesis for labeling cells in S phase) was added to the cultures for the final 30 min of incubation. Cells were then harvested, stained with both PI- and fluorescein-conjugated anti-BrdUrd Ab, and analyzed via FACS. Boxed populations represent cells in G1 phase, S phase, or G2 phase as labeled. Results are representative of three independent experiments.

of v-Abl inactivation (2). It is possible that in addition to decreasing apoptosis, IL-7 could also delay or prevent cell cycle arrest in these cells. To address this possibility, the distribution of cells in the different phases of the cell cycle was examined in ts-A-MuLV-transformed pre-B cells cultured at a nonpermissive temperature in the presence or absence of IL-7. The 103 cells were cultured with or without IL-7 and shifted to a nonpermissive temperature for 6, 12, or 18 h. BrdUrd (a marker of DNA synthesis for labeling cells in S phase) was added to the cultures for the final 30 min of incubation. Cells were then harvested, stained with PI- and fluorescein-conjugated anti-BrdUrd Ab, and analyzed via FACS to determine the percentage of cells in each phase of the cell cycle. An analysis of these FACS data demonstrates a progressive reduction in S-phase cells and an accumulation of cells in G1 phase (one diploid unit of DNA by PI staining, BrdUrd-negative) in cells cultured at a nonpermissive temperature (Fig. 3 and Table I). Adding IL-7 to the cultures decreased the reduction in S-phase cells and the accumulation of cells in G1 phase induced by temperature shift (Fig. 3 and Table I). IL-4 had no effect on the time-course of G1 arrest of these cells at a nonpermissive temperature (data not shown). These results demonstrate that IL-7 can decrease the cell cycle arrest induced by v-Abl inactivation in transformed pre-B cells.

**FIGURE 3.** IL-7 delays cell cycle arrest upon v-Abl inactivation in 103 cells. A. Cells were plated at a density of 0.5 × 10^6 cells/ml and incubated at 34°C, 39°C without IL-7, or 39°C with IL-7 for the indicated time intervals. BrdUrd (5 μg/ml) was added to the media for the final 30 min of culture. All samples were fixed and stained with both PI- and fluorescein-conjugated anti-BrdUrd Ab and analyzed via FACS. Boxed populations represent cells in G1 phase, S phase, or G2 phase as labeled. Results are representative of three independent experiments.

**IL-7 delays loss of c-Myc transcript levels on v-Abl inactivation**

One gene that may be mediating the proliferative signaling induced by A-MuLV in pre-B cell lines is c-myc. c-myc is a proto-oncogene that has been implicated in apoptosis, the regulation of cell cycle progression, and cellular differentiation (23). Transcription of c-myc is activated by multiple tyrosine kinases, and induction of c-Myc levels by v-Abl is thought to be required for A-MuLV-mediated transformation (24). Upon the shift of ts-A-MuLV transformed myeloid cells to a nonpermissive temperature, c-myc transcript levels have been shown to fall to undetectable levels (5). To determine whether IL-7 could affect the decreased expression of c-myc induced by v-Abl inactivation, ts-A-MuLV-transformed pre-B cells were cultured at a nonpermissive temperature for 20 h in the presence or absence of IL-7, and total RNA was analyzed by Northern blot. In cells cultured without IL-7, c-myc transcript levels are greatly decreased after 2 h at 39°C and are undetectable after 8 h. In contrast, when IL-7 is added to these cultures, c-myc transcript levels remain high even after 20 h at nonpermissive temperature (Fig. 4). These studies demonstrate that IL-7 can maintain c-Myc mRNA levels in transformed pre-B cells after loss of v-Abl activity.

**Activation of NF-κB and RAG genes at a nonpermissive temperature is not affected by IL-7**

Pre-B cells transformed by v-Abl remain in the pre-B stage of development and do not rearrange their Ig light chain loci (25). v-Abl-induced differentiation arrest has been studied in the 103/bcl-2-4 cell line, which was derived by stable transfection of 103 cells with human bcl-2 to enhance survival time at a nonpermissive temperature. These studies have demonstrated that the shift of 103/bcl-2-4 cells to a nonpermissive temperature leads to NF-κB activation, to increased levels of RAG-1 and RAG-2 transcripts, and to germline κ transcription (9, 26). In addition, these cells have been shown to rearrange the Ig κ locus. To determine whether IL-7 could alter the differentiation effects observed after v-Abl inactivation, 103/bcl-2-4 cells cultured at 39°C with and without IL-7 were transfected with their respective plasmids, and total RNA was isolated from these transfected cells treated with and without IL-7 at 39°C.

**FIGURE 4.** IL-7 delays loss of c-Myc mRNA following v-Abl inactivation in 103 cells. Total RNA was isolated from 103 cells cultured with or without IL-7 at the indicated time intervals following shift to a nonpermissive temperature and subjected to Northern blot analysis. The blot was probed for c-Myc and GAPDH expression as indicated.

**Table 1. Effect of IL-7 on cell cycle arrest of 103 cells at 39°C**

<table>
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<th>Hours at 39°C</th>
<th>IL-7</th>
<th>G1 phase</th>
<th>S phase</th>
<th>G2 phase</th>
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</thead>
<tbody>
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<td>-</td>
<td>50.8</td>
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<td>10.7</td>
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<td>+</td>
<td>60.6</td>
<td>22.8</td>
<td>15.2</td>
</tr>
</tbody>
</table>

*aQuantitation of boxed populations from Figure 3. Results are representative of three independent experiments.*
were examined for levels of activated NF-κB and RAG gene expression. EMSAs with nuclear extracts from these cells demonstrate that the same levels of NF-κB binding activity were detected when cells were cultured in the presence or absence of IL-7 (data not shown). Possible effects of IL-7 on RAG-1 or RAG-2 gene induction in this system were examined by Northern blot analysis. IL-7 appeared to have no effect on the increased levels of RAG-1 and RAG-2 transcripts detected when the cells were cultured at a nonpermissive temperature (Fig. 5). Levels of RAG-1 and RAG-2 transcripts upon temperature shift were also not altered by culture with IL-4 (data not shown). These experiments show that IL-7 did not alter the activation of NF-κB or the induction of RAG-1 and RAG-2 genes mediated by the inactivation of v-Abl kinase.

Stat5 activation in response to IL-7 is maintained at least 24 h after v-Abl inactivation

The ability of IL-7 and IL-4 to activate STAT5 and STAT6, respectively, in 103/bcl-2-4 cells has been demonstrated previously (10). The transient nature of the IL-7-mediated effects shown in the above experiments could be explained by a loss of responsiveness to IL-7 over time. To address this possibility, we examined the duration of IL-7-stimulated STAT5 activation in these cultures. The 103/bcl-2-4 cells were cultured at a nonpermissive temperature in the presence or absence of IL-7, and extracts from these cells were examined for STAT5 activation by EMSA (Fig. 6). After 6 h of culture at a nonpermissive temperature, activation of STAT5 was no longer detectable in samples cultured without IL-7. In contrast, STAT5 activation remained undiminished for at least 24 h in samples cultured at a nonpermissive temperature in the presence of IL-7. Similarly, STAT5 activation in response to IL-4 in 103/bcl-2-4 cells remained undiminished for at least 24 h at a nonpermissive temperature (data not shown). These data indicate that IL-7-mediated activation of STAT5 and IL-4-mediated activation of STAT6 are maintained for at least 24 h after v-Abl inactivation. Therefore, there is no observed correlation between IL-7-stimulated activation of STAT5 and IL-7-mediated effects on cell survival and cell cycle progression with regard to duration.

Discussion

The studies described above suggest that the activation of IL-7 signaling pathways is involved in the survival and proliferation of pre-B cells transformed by A-MuLV. Although corroborating previous results indicating that IL-7 signaling is not sufficient for complementation of v-Abl signaling (17, 18), the results go beyond these earlier studies, adding insights into the aspects of v-Abl-mediated transformation in which IL-7 may participate. The observation of constitutive STAT6 activation in A-MuLV transformed pre-B cells suggested a role for IL-4 as well. Although IL-4 can induce long-term (>24 h) activation of STAT6 in the ts-A-MuLV-transformed pre-B cell line used, the above experiments have shown no function of IL-4 in the survival, proliferation, or differentiation of these cells. The complete lack of effect of IL-4 in our studies is somewhat surprising, considering IL-4 and IL-7 activate several of the same signaling molecules, including JAK1, JAK3, insulin receptor substrate-1, insulin receptor substrate-2, and phosphatidylinositol 3-kinase (27–29). The different activities of the two cytokines in our system suggest that the observed effects of IL-7 depend upon a signal that is not delivered by IL-4, such as STAT5 activation. In support of such a possibility, a recent study by Zamorano et al. has shown that a truncated IL-2R β chain can support survival in response to IL-2 in 32D cells when it contains a STAT5 activation site but not when it contains a STAT6 activation site (30).

One difference observed between the cellular events induced by IL-7 and those induced by v-Abl is seen in the duration of activation. Although v-Abl induces a high level expression of bcl-2, bcl-xL, and c-myc indefinitely, the induction of these genes by IL-7 declines in <24 h. This kinetic difference correlates with the duration of cell survival and proliferation mediated by v-Abl and IL-7, respectively. It is possible that a signal activated by v-Abl but not by IL-7 is necessary for continued stimulation of signaling pathways downstream from the IL-7R. Another possibility is that ligand-dependent signaling through the IL-7R complex activates negative regulatory mechanisms, such as the recruitment of phosphatases, suppressor of cytokine signaling proteins (31–33), or receptor modulation, which are not activated by the initiation of IL-7 signaling by v-Abl. The activation of STAT5 in response to IL-7 at a nonpermissive temperature remains high at timepoints when cell survival and proliferation have already begun to decline, indicating that STAT5 activation is not sufficient for the observed effects of IL-7 on cell survival and cell cycle progression. It is of interest that as B cell precursors mature, expression of the IL-7R is lost (34), suggesting that differentiation and a loss of responsiveness to IL-7 in ts-A-MuLV-transformed pre-B cells may be linked events.

The arrest of cellular differentiation is a common feature of transformed cells and is thought to be an important component of oncogenesis. In the cells used in our studies, v-Abl arrests differentiation at the pre-B cell stage. Differentiation in the B cell lineage can be followed by analysis of surface molecule expression and rearrangement status of the Ig locus (35). Prior studies have suggested that IL-7 can decrease levels of RAG-1 and RAG-2 in pre-B cells (36). In contrast, other studies have supported a role for IL-7 signaling in the induction of Ig rearrangement (37). In our studies, IL-7 had no effect on the induction of RAG-1 and RAG-2 genes upon v-Abl inactivation. In addition to altering RAG-1 and RAG-2 gene expression, v-Abl activity is associated with inactivation of the κ intronic enhancer. The repression of the κ intronic
enhancement in v-Abl-transformed pre-B cells has been shown to be due to stabilization of IκB in these cells, resulting in an inhibition of NF-κB/Rel activity (26). In our experiments, IL-7 failed to maintain NF-κB/Rel inhibition upon v-Abl inactivation. These results suggest that although v-abl can arrest differentiation in pre-B cells, IL-7 is not sufficient to prevent differentiation from the pre-B cell stage.

In contrast to the lack of effect of IL-7 on the induction of differentiation, loss of bcl-2 and bcl-xL expression upon v-Abl kinase inactivation was delayed by IL-7. The regulation of bcl-2 family members by v-Abl has also recently been observed in a pre-mast cell line (22) and is interesting in light of the prior observation that bcl-2 is essential for BCR-ABL-mediated transformation (21). Regulation of Bcl-2 levels by IL-7 has been demonstrated previously in several cell types, including pro-B cells, NK cells, T cell progenitors, and mature T cells (38–43). In the T cell lineage, the regulation of Bcl-2 levels by IL-7 is essential for normal T cell development. Although the above data show a correlation between cell survival and levels of Bcl-2 and Bcl-xL, whether bcl-2 family members are required for transformation by v-Abl remains to be established. Overexpression of human bcl-2 in the ts-A-MuLV-transformed cell line 103 has been shown previously to delay apoptosis secondary to loss of v-Abl activity (9). Taken together, these results suggest that upon v-Abl inactivation in ts-A-MuLV-transformed pre-B cells, the observed delay in apoptosis in cells cultured with IL-7 is, at least in part, mediated by IL-7-stimulated regulation of either bcl-2, bcl-xL, or a combination of these molecules.

Previous studies by Merino et al. determined the t1/2 of Bcl-2 within a B cell line to be ~10 h; in our experiments, the t1/2 of Bcl-2 was closer to 6 h (44). Whereas the measurements of Merino et al. were made in the absence of any apoptotic stimuli, our system included an apoptotic stimulus, namely v-Abl inactivation. These results provide evidence for active degradation of Bcl-2 in cells subjected to certain apoptotic stimuli. One potential mechanism for this degradation has been revealed in recent work showing cleavage of Bcl-2 by activated caspases in cells undergoing apoptosis (45).

Although the overexpression of antiapoptotic molecules such as Bcl-2 and Bcl-xL may promote oncogenesis, additional signals appear to be required for cellular transformation. One oncogene that can cooperate with bcl-2 for transformation is c-myc. Overexpression of c-myc and bcl-2 has been shown to immortalize pre-B cells (46, 47). c-myc has also been implicated as an important factor in transformation by oncogenic forms of c-Abl. v-Abl activates transcription of c-myc in myeloid cells (5), and transformation of pre-B cells by both v-Abl and BCR-ABL can be blocked by expression of transdominant negative c-Myc (24). c-myc transcription is also induced by IL-7 in pre-B cells (48). Our results demonstrate that when v-Abl-induced c-myc transcription is inactivated in pre-B cells, IL-7 treatment can maintain levels of c-myc and also delay the loss of proliferative activity of the cells, transiently substituting for v-Abl in these functions. IL-7 and v-Abl both regulate the cell cycle during G1 phase, supporting the possibility that they may use similar signaling pathways in this regulation (2, 49).

Several recent studies have demonstrated the activation of cyto kinase signaling molecules in different forms of malignancy. Although the oncogenes BCR-Abl, v-src, and v-abl have all been shown to activate STAT molecules in transformed cells (10–13, 50–52), the functional significance of cytokine signaling pathways in oncogenesis is not known. In this study, we have demonstrated that signaling through IL-7 can reduce the cell cycle arrest and apoptosis that ts-A-MuLV-transformed pre-B cells undergo with the loss of v-Abl kinase activity.

IL-7 RECONSTITUTES v-Abl-MEDIATED SIGNALING

Acknowledgments

We thank Dr. Robert Coffman for murine rIL-4, Dr. Naomi Rosenberg for the 103 and 103/bcl-2-4 cell lines, Dr. Tim McDonnell for antisera to Bax, Dr. Frederick Alt for RAG-1 and RAG-2 probes, and Dr. Kathryn Calame for cDNA for the c-Myc probe. We also thank Nika Danial for critical reading of the manuscript.

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