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Abelson Virus Abrogation of Interleukin-3 Dependence in a Lymphoid Cell Line

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Among several tyrosine-protein kinases, only v-*abl* could abrogate interleukin 3 dependence of a lymphoblastoid cell line; v-*src* and v-*fps* proteins gave partial or no interleukin 3 independence, respectively. Lymphokine independence was achieved via a nonautocrine mechanism. Direct involvement of c-*myc* in this process was not evident.

Nonlymphoid hemopoietic cells infected with Abelson murine leukemia virus (A-MuLV) have been reported to lose their requirement for interleukin 3 (IL-3) for growth in vitro (2, 6, 8). Lymphoid cells are the usual target of A-MuLV in vivo (10). We have extended our knowledge from earlier observations to an IL-3-dependent, lymphoid precursor cell line, Ba/F3, and have compared the abilities of different viruses encoding tyrosine-protein kinases to affect the IL-3 requirement of Ba/F3 cells. In addition, we have examined the role of c-*myc* gene regulation in this process.

Clone Ba/F3 was derived from murine bone marrow. Like similar clones, it expresses B-cell-specific surface glycoprotein B220 but has unrearranged immunoglobulin genes. It lacks T-cell (Thy1, Lyt), myeloid (MAC-1, Ia), and mature B-cell (Ig, Ia) antigens and strictly requires IL-3 for growth in vitro (7).

We infected Ba/F3 cells with A-MuLV, helper Moloney murine leukemia virus (Mo-MuLV), or an A-MuLV derivative (V-SAB) in which A-MuLV *gag* sequences have been replaced with a 5′ fragment of the *src* gene (4). Cells were grown for 3 days in RPMI medium–10% fetal calf serum–10% WEHI-3 conditioned medium (as a source of IL-3) (7) and then were transferred to medium lacking IL-3. Uninfected and Mo-MuLV-infected cells died within 48 h without IL-3 supplementation. In contrast, 0.1% of the cells treated with A-MuLV or V-SAB viruses survived. Mass cultures were obtained within 2 weeks, and single cell clones were derived. The low percentage of IL-3-independent cells after A-MuLV or V-SAB infection reflects their poor infectibility. When Ba/F3 cells were infected with the *zip-neo* retrovirus, which confers G418 resistance (1), only 0.1% of the cells survived G418 selection in IL-3-containing medium. IL-3-independent cell clones expressed the transforming protein of A-MuLV or V-SAB viruses detected by an in vitro kinase assay (Fig. 1A, lanes b and c). For this experiment, extracts of infected clones were immunoprecipitated with specific antisera, incubated with [γ-^{32}P]ATP, and electrophoresed through polyacrylamide gels. Because of residual nucleic acids in the extract, P160$^{gag\text{-}abl}$ appeared as a diffuse band near the top of the gel (Fig. 1A, lane b). This was also observed with different cell types infected with A-MuLV (data not shown).

Because v-*abl* belongs to the protein-tyrosine kinase oncogene family (12, 13), we tested the effects of other members on the IL-3 requirement of Ba/F3 cells. V-SRC, V-GΔSRC, and V-GFPS recombinant viruses carry the *src* gene in a Mo-MuLV vector (V-SRC) or part of the *src* or *fps* genes fused to *gag* of A-MuLV (V-GΔSRC and V-GFPS, respectively) (4).

Infections with viruses Mo-MuLV and A-MuLV were carried out in parallel. Two days after the removal of IL-3 from medium, no surviving cells were observed in the cultures infected with Mo-MULV or V-GFPS. By contrast, equal numbers of surviving cells were detected in the A-MuLV, V-SRC, and V-GΔSRC cultures, whereas clones of A-MuLV-infected cells rapidly divided in the absence of IL-3. Clones of V-SRC- and V-GΔSRC-infected cells grew very poorly and appeared unhealthy. Rapid proliferation was achieved only after adding back a small amount of IL-3. All single cell clones derived from cultures infected with V-SRC and V-GΔSRC expressed p60^{src} and P78$^{gag\text{-}\Delta src}$, respectively (Fig. 1B, lanes b and c; additional data not shown). [^{35}S]methionine labeling showed that these proteins were expressed at levels similar to those of P160$^{gag\text{-}abl}$ (data not shown).

To allow selection of cells infected with a v-*fps*-containing virus, we inserted the coding sequence of V-GFPS into the DOL vector (provided by A. Korman) upstream of the *neo* gene driven by a simian virus 40 promoter. The resulting construct, pV-GFOL, was cotransfected with Mo-MuLV DNA onto NIH 3T3 cells, and foci were isolated in the presence of G418. Recovered virus from the best producer clone was used to infect Ba/F3 cells. After G418 selection in the presence of IL-3, some of the resistant cells were grown in the absence of IL-3. These cells died within 2 days. Cells from the same culture kept in IL-3 expressed P125$^{gag\text{-}fps}$, albeit at a lower level than in the V-GFOL producer NIH 3T3 clone (Fig. 1C, lanes b and c). Although P125$^{gag\text{-}fps}$ expression is usually low in fibroblasts infected with V-GFOL virus, 5 to 10% of infected cells expressed high levels of the transforming protein (Fig. 1C, lane b; additional data not shown). To attempt selection for high expression of P125$^{gag\text{-}fps}$ in V-GFOL-infected Ba/F3 cells, we grew them without IL-3 for enough time periods to kill the majority of cells. After allowing the recovery of surviving cells in IL-3, we measured expression of P125$^{gag\text{-}fps}$ and the IL-3 requirement of the cells. In two separate experiments, the recovered population behaved like the parental culture (data not shown). Thus, the v-*fps* oncogene, in contrast to v-*abl* and

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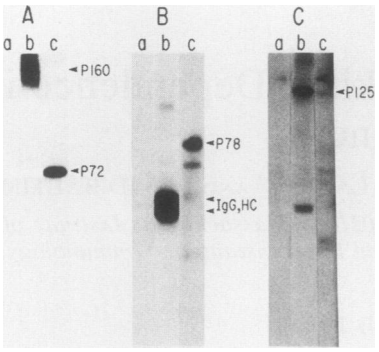

FIG. 1. In vitro kinase assay of immunoprecipitated viral proteins. Extracts of uninfected, v-*abl*-, v-*src*-, and v-*fps*-infected Ba/F3 clones were immunoprecipitated with the indicated antisera, and kinase activity was assayed as previously described (5). (A) Anti-*abl* 587 antiserum (11); lanes: a, Ba/F3; b, A-MuLV Ba/F3; c, V-SAB Ba/F3. (B) Anti-Rous sarcoma virus, TBR antiserum (v-*src* specific) (lanes a and b) and anti-MuLV antiserum (lane c); lanes: a, Ba/F3; b, V-SRC Ba/F3 (only phosphorylation of the immunoglobulin heavy chain by $p60^{v\text{-}src}$ was observed with tumor-bearing rabbit antiserum); c, V-GΔSRV Ba/F3. (C) Anti-MuLV antiserum; lanes: a, Ba/F3; b, V-GFOL NIH 3T3; c, V-GFOL Ba/F3. Panels A, B, and C represent different gels.

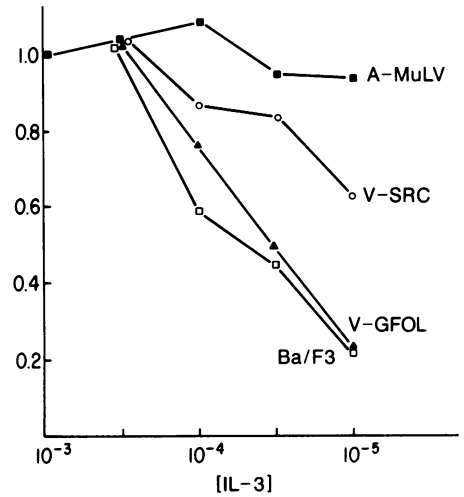

FIG. 2. Proliferation assay. Uninfected or virus-infected Ba/F3 cells (10^4 cells per well) were cultured for 24 h with IL-3 at the indicated dilutions of a stock solution. Proliferation was then measured in a colorimetric assay as previously described (5). Measurements have been normalized by dividing the optical density at a given IL-3 dilution by the density obtained at a 10^{-3} dilution of IL-3.

v-*src*, did not provide any growth advantage to Ba/F3 cells in the absence of IL-3 (Table 1).

As an illustration of the differences in uninfected and A-MuLV-, V-SRC-, and V-GFOL-infected Ba/F3 cells, we titrated their IL-3 requirements. Cell proliferation was monitored by colorimetric assay (5) after a 24-h incubation (Fig. 2). A-MuLV-infected cells were totally IL-3 independent for growth. V-SRC-infected cell clones displayed an intermediate proliferation profile. V-GFOL-infected cells behaved similarly to uninfected cells (they were assayed in a separate experiment). These results are consistent with earlier observations (Table 1).

The mechanisms by which A-MuLV confers IL-3 independence remain uncertain. In agreement with other reports (2, 6, 8), we could rule out autocrine stimulation in virus-infected cells. Poly(A)-containing RNA (2 μg) from infected and uninfected Ba/F3 cells and WEHI cells was electrophoresed, transferred to nitrocellulose, and hybridized to an IL-3 specific probe (Fig. 3). Only WEHI cells expressed the 1-kilobase IL-3 mRNA (lane b) (14). Thus, IL-3 independence in infected Ba/F3 cells did not result from endogenous synthesis of IL-3. Furthermore, neither conditioned medium nor freeze-thaw lysates from A-MuLV infectants supported growth of uninfected Ba/F3 cells (data not shown). In addition, we found the majority of IL-3 receptors in infected cells available for binding and not occupied by a virus-induced growth factor that could compete for the IL-3 receptor (data not shown).

v-*abl* might relieve IL-3 dependence of Ba/F3 cells by inducing differentiation, presumably along the B-cell lineage.

TABLE 1. IL-3 requirement of Ba/F3 cells after viral infection[a]

Virus[b]	Transforming gene	Titer[c]	Transforming protein	IL-3 independence
Mo-MuLV	—[d]	1×10^7	—	No
A-MuLV	v-*abl*	1×10^6	$P160^{gag\text{-}abl}$	Total
V-SAB	v-*abl*	1×10^6	$P72^{src\text{-}abl}$	Total
V-SRC	v-*src*	5×10^5	$p60^{src}$	Partial
V-GΔSRC	v-Δ*src*	5×10^6	$P78^{gag\text{-}\Delta src}$	Partial
V-GFPS	v-*fps*	5×10^5	$P125^{gag\text{-}fps}$	No
Zip-*neo*[e]	—	1×10^6	—	No
V-GFOL[e]	v-*fps*	5×10^4	$P125^{gag\text{-}fps}$	No

[a] Ba/F3 cells (10^6) were suspended in 3 ml of growth medium containing 10% of WEHI-conditioned medium. Virus (1 ml) was added to infect cultures. Cells were incubated at 37°C overnight, washed, and replated in 10 ml of growth medium containing 10% WEHI-conditioned medium. After 24 h, cells were washed twice and suspended in 10 ml of growth medium without a source of IL-3. Medium was changed every 3 days, and cells were kept at a density of at least 5×10^3/ml.

[b] All replication-defective viruses were pseudotyped with Mo-MuLV.

[c] Titers were measured on NIH 3T3 or XC cells. They are expressed as PFU, focus-forming units, or G418 CFU per milliliter.

[d] —, None.

[e] For viruses containing the *neo* gene, the same infection protocol was used, but G418 selection was applied first (1.5 mg of G418 per ml), in the presence of IL-3.

FIG. 3. Absence of IL-3 mRNA in V-SRC- or A-MuLV-infected Ba/F3. Glyoxal-treated, poly(A)-containing RNA (4 μg) were electrophoresed through a 1% agarose gel, transferred to nitrocellulose, and hybridized to an IL-3 cDNA probe. Lanes: a, Ba/F3; b, WEHI; c, V-SRC Ba/F3; d, A-MuLV Ba/F3.

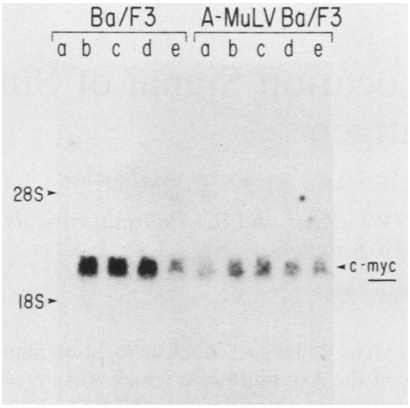

FIG. 4. Effect of IL-3 or A-MuLV infection on c-*myc* mRNA in BA/F3 cells. Total RNA was isolated from uninfected or A-MuLV-infected Ba/F3 cells at the indicated times after IL-3 addition. For each time point, 20 μg of RNA was treated with glyoxal, electrophoresed through a 1% agarose gel, transferred to nitrocellulose, and hybridized to a mouse c-*myc* cDNA probe. Rehybridizing the blot with a β2-microglobulin probe revealed an equal amount of RNA in each lane (not shown). Migration of 28S and 18S rRNA is shown. Lanes at hours indicated: a, 0; b, 1; c, 2; d, 6; e, 24.

No evidence of immunoglobulin heavy-chain rearrangement was detected (3), however, in DNA from v-*abl*-infected clones (data not shown).

Transformation by a virus expressing v-*myc* alleviated IL-2 or IL-3 requirements in lymphokine-dependent cultured cells (9). To examine whether IL-3 independence is achieved by v-*abl* via c-*myc* deregulation, we studied regulation of c-*myc* expression in uninfected and A-MuLV-infected Ba/F3 cells. Cultures were maintained for 12 h in the absence of IL-3 (more than 75% of cells were still alive at that time). IL-3 was then added, total RNA was isolated at various time points, and c-*myc* transcripts were analyzed by Northern hybridization (Fig. 4). In the absence of IL-3, no c-*myc* transcription was detected in the nonproliferating, uninfected cells. Addition of IL-3 caused a transient increase in c-*myc* RNA that leveled off with time. In infected cells, IL-3 caused a small increase in c-*myc* RNA, after a time course similar to that seen for uninfected cells. In IL-3-deprived, A-MuLV-infected cells, we detected a low level of c-*myc* RNA. This increase may reflect v-*abl* action on c-*myc* expression or cell proliferation. Thus, c-*myc* RNA levels respond to A-MuLV infection and to IL-3 in Ba/F3 cells, but this response could not be directly uncoupled from proliferation. Other investigators have shown that v-*myc*-infected, IL-3-independent cells do not have detectable levels of c-*myc* RNA (9), suggesting that the presence of the v-*myc* protein could substitute for c-*myc* in growth. We do not have direct evidence that v-*abl* causes IL-3 independence via a specific increase in the c-*myc* message, although we cannot rule it out.

That v-*abl* acts on a wide range of IL-3-dependent, hemopoietic cells is well documented (2, 6, 8). v-*abl* must act on a common denominator shared by these cells, possibly by subverting the same pathway used by IL-3. It is therefore important to address the substrate specificity of v-*abl* protein among tyrosine kinases as a key to unlocking the cellular events leading to IL-3 independence.

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