Splenic Accumulation of Interleukin-3-Dependent Hematopoietic Cells in Friend Erythroleukemia

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Interleukin-3-dependent hematopoietic stem cells commonly accumulate in spleens of mice infected with leukemia viruses. To study their origins, a molecularly tagged helper-free Friend spleen focus-forming virus was used to produce erythroleukemias. Uninfected interleukin-3-dependent basophil-mast cell progenitors coproliferated amidst the spleen focus-forming virus-infected leukemic cells. Splenic proliferation of normal stem cells is apparently a host response to leukemogenesis, and we propose that it may contribute to certain retroviral diseases.

Preleukemic neoplasms form in the spleens of mice infected with murine leukemia viruses (MuLVs), and they contain a variety of hematopoietic precursor cells, including cells that can proliferate in culture only in the presence of interleukin-3 (IL-3) (3, 8-12, 15, 16, 25). These infected hematopoietic stem cells accumulate in the spleens regardless of whether the MuLVs cause thymic lymphoma or erythroleukemia (3, 10-12, 15, 16, 25), but they do not accumulate in genetically resistant mice (8-11) or in mice infected with nonpathogenic MuLVs (3). Consequently, it has been proposed that these stem cell neoplasms are prerequisites for the development of MuLV-induced cancers and that they constitute in some cases the target cell pool for malignant transformation (3, 8-12, 15, 16, 25). However, it has not been shown whether MuLV infection of these cells is necessary for their proliferation and whether they are progenitors, accessories, or by-products of leukemogenesis. Their IL-3 dependency can be abrogated by superinfections with Abelson MuLV (15, 18) or with viruses that contain the *v-myc* oncogene (20).

Recently, we and others (1, 2, 24, 27, 28) analyzed erythroleukemias induced in mice by injection of helper-free Friend spleen focus-forming viruses (SFFVs). We used a modified polycythemia-inducing molecular clone (called SVSF) that contained a small 215-base-pair tag of simian virus 40 (SV40) DNA inserted into its nonfunctional *pol* gene (24). This modification enabled us to identify infected cells by Southern blot analyses with SV40 DNA as the hybridization probe. Although most erythroblasts infected with helper-free SVSF proliferated only briefly (7 to 12 days) before expressing a commitment to differentiate that caused transient polycythemia *in vivo*, rare infected cells that occurred in a minority of the mice proliferated by 26 days to form immortalized transplantable leukemias (24). Each transplantable leukemia that was examined was clonal and contained a single SVSF provirus (24). Immortalization involved inhibition of the commitment to differentiate; mice with clonal erythroleukemias consequently became anemic.

During the above studies, we attempted to culture cells from the leukemic spleens (see Table 1 for a description of

the cell lines). In this manner, IL-3-dependent cell lines were routinely obtained. The morphology of these cell lines indicated that they were basophil-mast cells (e.g., see Fig. 1A). Thus, they showed intense basophilic granules when stained with Wright stain and metachromasia diagnostic of basophil-mast cells when stained with toluidine blue. They were negative when stained for peroxidase, for butyrate esterase, or for glycogen with periodic acid-Schiff reagent (29). Cells derived from one leukemia (IP/IR) were culturable in the absence of IL-3 (Table 1). IP/IR resembles other Friend leukemia cell lines (Fig. 1B), and it can be induced to synthesize hemoglobin by treatment with 1% dimethyl sulfoxide.

The transplantable leukemias obtained in these studies contained the SFFV-encoded glycoprotein gp55 (4, 21) but not the gp90 and gp70 glycoproteins that would be indicative of helper virus contamination (Fig. 2, lanes 2 to 4). In contrast, IL-3-dependent basophil-mast cells cultured from leukemic spleens lacked gp55 (Fig. 2, lanes 5 to 8). Southern blot analyses of DNAs indicated the presence of the 2.0-kilobase tagged *Eco*RI fragment of the SVSF provirus in the transplantable erythroleukemias (Fig. 3, lane 3) but not in the DNAs of normal spleen cells or of IL-3-dependent basophil-mast cells (Fig. 3, lanes 1 and 2, respectively).

The IL-3-dependent basophil-mast cells did not form tumors in syngeneic secondary recipient mice (Table 1). Consistent with earlier studies (10-16), these cells stopped proliferating and died when deprived of IL-3. This requirement could be supplied by WEHI-3-conditioned medium or by purified recombinant IL-3. IL-3 dependency of these cell lines was not eliminated by growth in medium conditioned by Friend erythroleukemia cells or by addition of a partially purified soluble form of gp55 (this form of gp55 is spontaneously shed from surfaces of SFFV-infected cells into their culture media [19]). The IL-3-dependent cells grew slowly (generation time approximately 2 days), and they became senescent after several months in culture. Although we have reproducibly grown IL-3-dependent cell lines from spleens of mice injected with transplantable erythroleukemias (Table 1), we and others (11, 12, 16) have been unable to isolate such cells from normal spleens. Injection of mice with phenylhydrazine causes anemia associated with splenic enlargement and erythropoiesis (5). We were also unable to isolate IL-3-dependent cell lines from the latter spleens. Possibly, changes in the methods for dispersal of spleen cells

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FIG. 1. Morphology of cell lines developed in this study. Cells were deposited onto slides with a cytocentrifuge and were stained with Wright stain. (A) Typical example of an IL-3-dependent cell line (H-29B). The cells contain numerous darkly stained basophilic granules. (B) IP/IR leukemia cells. The cells often contain blebs and have a morphology similar to that of other erythroleukemias. Bar, 10 μ m.

or in the culture conditions could influence the isolation of these cell lines.

Although IL-3 is required for proliferation and differentiation of most primitive hematopoietic stem cells, basophil-mast cells tend to predominate in long-term cultures of normal hematopoietic cells grown in IL-3 (10, 14, 18, 30). It has been proposed that this may occur because the other cell lineages grow less rapidly than basophil-mast cells, perhaps because they differentiate and lose their self-renewal capa-

TABLE 1. Cell lines developed from infected mice^a

Cell line	IL-3 dependency	gp55	Transplantable
E (26-A)	+	—	—
F (26-B)	+	—	—
H (29-B)	+	—	—
I (29-C)	+	—	—
IP/IR (27-4D)	—	+	+

^a The first four cell lines were derived from the previously described leukemic spleens E, F, H, and I, respectively (24), whereas IP/IR was from another leukemia harvested 27 days after SVSF virus infection. Female NIH Swiss mice (4 to 6 weeks old) were injected with helper-free SVSF (the tagged SFFV) as previously described (24). Persistently enlarged spleens (1.5 to 2 g compared with the normal 0.10 to 0.15 g) developed in approximately 10 to 20% of the mice by 26 to 29 days. These spleens were harvested and examined for SVSF provirus by Southern blot analysis, for expression of gp55 by protein blotting, and for ability to induce tumors on the omenta of irradiated mice. All enlarged spleens were positive by these three assays and lacked helper virus contamination. Spleen cells (5×10^6 cells) were also passaged by intravenous injection into unirradiated secondary recipients, which all developed splenomegaly by 2 to 3 weeks. Splenic tissue from these secondary recipients was more easily dispersed to form single-cell suspensions, and these cells were used for culturing in Dulbecco modified Eagle medium plus 20% fetal calf serum and 10% WEHI-3 (10, 12)-conditioned medium. The first four cell lines in the table (E, F, H, I) grew out within several weeks. The IP/IR cell line derived from the primary spleen of an SVSF-infected mouse. Dispersed cells were first cultured for 17 days in Dulbecco modified Eagle medium containing 10% fetal calf serum. Nearly all the cells died, and 10^5 of the remaining cells were injected intraperitoneally into sublethally irradiated mice. After 3 weeks, an enlarged spleen from one mouse was dispersed and cultured as described above. At that time, the IP/IR cells grew vigorously, with cell doubling every 22 h.

FIG. 2. Electrophoresis and immunoblotting of virus-encoded glycoproteins from leukemic spleens and from the corresponding derived IL-3-dependent cell lines. The leukemias were from mice infected with the tagged SFFV (see text). Equivalent concentrations of splenic tissue and of the derived IL-3-dependent cell lines were analyzed by immunoblotting with an antibody made to Friend MuLV gp70 that cross-reacts with gp55, as described elsewhere (4, 21, 24). Lane 1, Friend 745 erythroleukemia cells. This standard cell line contains the gPr90 glycoproteins encoded by Friend MuLV as well as the gp55 and gp55^P glycoproteins encoded by SFFV. Lanes 2 to 4, Tissue from the leukemic spleens E (26-A), H (29-B), and I (29-C), respectively. These leukemic spleens were from mice infected with helper-free SVSF virus. As expected, they contain gp55 but not any helper virus glycoproteins. Lanes 5 to 8, IL-3-dependent cell lines derived from the SVSF-infected leukemic spleens E (26-A), F (26-B), H (29-B), and I (29-C), respectively. These IL-3-dependent cell lines lack virus-encoded glycoproteins.

FIG. 3. Southern blot analysis of DNA from spleens and from a corresponding derived IL-3-dependent cell line. The high-molecular-weight DNAs were digested with the restriction endonuclease *Eco*RI, and the electrophoresed fragments were then blotted onto nitrocellulose and probed with the nick-translated SV40 DNA as described elsewhere (24). The tagged SFFV provirus contains *Eco*RI sites on both sides of the 215-base-pair SV40 insert, so that the fragmented provirus should yield a 2.0-kilobase fragment that hybridizes with the SV40 probe (24). Lanes: 1, normal spleen DNA; 2, DNA from the IL-3-dependent cell line H (29-B); 3, DNA from the leukemic spleen H (29-B). Although the leukemic spleen contains an SVSF provirus, as indicated by the 2.0-kilobase hybridizing fragment, the corresponding derivative IL-3-dependent cell line (lane 2) and the normal spleen (lane 1) lack any SVSF provirus. kb, Kilobase.

bilities or become dependent on factors other than IL-3 (8–10, 23). Consistent with these ideas, our IL-3-dependent cell cultures initially contained some monocytes and neutrophils. Accordingly, although we isolated only basophil-mast cell lines, our results suggest that uninfected IL-3-dependent hematopoietic progenitor cells were accumulated in the leukemic spleens.

We conclude that splenic recruitment of IL-3-dependent hematopoietic progenitors can occur owing to an extrinsic factor(s) rather than because of infection or evident abnormality within this cell population. In addition, erythroleukemia cells do not seem to release any stable factor that can abrogate IL-3 dependency of basophil-mast cells. Conceivably, alterations in the splenic or bone marrow microenvironments or in hormonal factors might cause migration and proliferation of these cells in the spleens. IL-3 is produced by antigenically activated T lymphocytes, and it is possible that an immune response to an SVSF-encoded protein(s) could stimulate IL-3 release and splenic proliferation of stem cells (8–10).

Presumably, a similar host response pathway may be involved in initiating splenic proliferation of IL-3-dependent stem cells in leukemias induced by replication-competent MuLVs. For MuLVs capable of transforming these cells (8–10), this host response would contribute to pathogenesis by expanding the target cell population. Furthermore, because retroviral infection and progeny release both require

cell proliferation (7, 17, 22, 26), a host response that caused cell replication would greatly increase virus production and the probability of leukemic transformation by the proviral insertion mechanism (6). Similarly, immunogenic MuLVs can preferentially transform lymphocytes that contain antigen receptors specific for the virus (13). Mice that are immunologically nonresponsive do not develop these lymphomas (8, 11). In all cases, MuLV leukemogenesis therefore seems targeted to cell populations that are induced to proliferate by a host response pathway rather than by a virus-produced cellular abnormality. It is conceivable that the IL-3-dependent hematopoietic stem cells could also have inhibitory effects on the development of some retroviral diseases.

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