Effects of Purified Iron-Saturated Human Lactoferrin on Spleen Morphology in Mice Infected With Friend Virus Complex

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The present report describes effects of lactoferrin treatment on the development of erythroleukemia in the spleen of mice infected with Friend virus complex (FVC). Lactoferrin (LF) treatment was carried out in mice for up to 2 weeks at a total dose of 200 μg per mouse. The treatment was started at 1) Days 7 and 14 prior to viral infection and 2) Days 0, 1, 3, 7, and 11 after viral infection. Spleens were analyzed 14 days after viral infection. In mice whose treatment was initiated at Days 0 and 1, few leukemic cells were present in the spleen. Most of them appeared in clumps in the red pulp. No leukemic cells were seen in the white pulp. The white pulp was greatly enlarged. In mice whose treatment was initiated at Day 3, leukemic cells began to spread out in the red pulp and encroached upon the white pulp. The white pulp was enlarged and clearly visible. In mice whose treatment was initiated at Days 7 and 11, many leukemic cells were present in the red pulp. The white pulp was infiltrated by leukemic cells and became less discernible. The morphologic features of the spleen in mice whose treatment was initiated at Day 7 or 14 prior to viral infection were similar to those of untreated groups. Leukemic cells not only filled most of the cordal space in the red pulp but also invaded the white pulp. Many leukemic cells were seen in venous sinuses. When infected mice responded to LF treatment, the general architecture of the red pulp remained intact and the white pulp was enlarged but not infiltrated by leukemic cells. (Am J Pathol 1987, 126:285–292

INJECTION of Friend virus complex (FVC) containing a replication-defective spleen focus-forming virus (SFFV) and a replicating helper virus of Friend murine leukemia (F-MuLV) into mice induces erythroleukemia within 2 weeks and causes a rapid enlargement of the spleen. Mice die within 3 months after viral infection.[1] Many deaths result from splenic rupture.[2] Purified iron-saturated human lactoferrin has a protective effect and prolongs survival time of Friend virus-infected mice when lactoferrin treatments are initiated at Days 0–3 after viral injection, and fewer SFFVs are observed in the spleens of lactoferrin-treated mice than in those of untreated mice.[1] The present study describes the effects of lactoferrin treatment on the development of erythroleukemia in the spleens of mice infected with FVC.

Materials and Methods

Four to 6-week-old female BDF_1 (C57BL/B6 × DBA/2) mice were used in this study. The FVC contains a replication defective SFFV and F-MuLV (clone 201) and was grown in NIH 3T3 or BALB/c 373 mouse embryo fibroblast cell line cells as described.[3] The FVC was kindly provided by Dr. D. H.

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Troxler and Dr. F. M. Scolnick. The stock virus contained 10^6 plague-forming units (PFU)/ml and 10^5 spleen focus-forming units (SFFU)/ml.[3,4] The FVC was diluted at a 1 : 5 ratio in sterile pyrogen-free saline, and 0.5 ml of this virus-containing medium was injected intravenously into each mouse. Control mice received no virus, but the same volume of sterile pyrogen-free saline.

Lactoferrin (LF) obtained from Calbiochem (San Diego, CA) was purified from human breast milk[5] and fully iron-saturated.[6] Iron saturation was measured at an absorbance maximum of 457–460 nm. Lactoferrin did not contain detectable endotoxin, as measured with the E-Toxate test (Sigma Chemical Co., St. Louis, Mo). Lactoferrin was given intravenously at a total dose of 200 μg per mouse. The dosage schedule is shown in Table 1. Control mice were inoculated with sterile pyrogen-free saline at the same volume and in a schedule similar to that for mice given lactoferrin.

Three complete experiments were performed. Each experiment consisted of eight LF-treated groups and controls. Each group was composed of 6 mice. The data given are for one representative experiment. For morphologic studies, four spleens per group were used. Spleens were removed 14 days after viral infection, weighed, and processed for light and electron microscopy. Half of each spleen was used for light microscopy and the other half for electron microscopy. For light microscopy, the tissue was fixed in 10% formalin, dehydrated in ethanol, and embedded in paraffin. Sections were stained with hematoxylin and eosin. For electron microscopy, spleens cut into small blocks (0.5-mm) were fixed in glutaraldehyde–paraformaldehyde mixture[7] for 12 hours, washed, and postfixed in 1% osmium tetroxide in cacodylate buffer. The tissue was then dehydrated in ethanol and embedded in Epon. Thin sections were stained in aqueous uranyl acetate and lead citrate and studied under the electron microscope (Philips 301). For light microscopy, five random sections from each spleen were examined. For the identification of virus particles in each spleen, four grids from two tissue blocks were studied under the electron microscope.

Results

Spleen weights of FVC-infected mice without LF treatment or whose lactoferrin treatment was initiated at Days 7 or 14 prior to viral infection were 2 times (2.07 ± 0.40 g) larger than those of FVC-infected mice whose LF treatment was initiated at Day 0 (0.92 ± 0.21 g) and 20 times larger than those of mice (0.08 ± 0.01 g) not given FVC or LF. When LF treatment was delayed after viral infection, the size of the spleen increased. That is, the spleens of mice whose LF treatment was initiated at Day 7 were larger than those of mice whose treatment was initiated at Day 3, which in turn were larger than those of mice whose treatment was initiated at Day 0.[1]

The morphologic features of the spleens were investigated 14 days after viral infection. In mice infected with FVC and not given LF treatment, leukemic cells not only filled most of the cordal space in the red pulp but also invaded the white pulp (Figures 1 and 2). Many leukemic cells were seen in venous sinuses (Figure 3). Budding of virus particles were frequently observed at the surface of leukemic cells (Figure 4). In mice whose LF treatment was initiated at Days 0 and 1, few leukemic cells were present in the spleen. Most of them appeared in clumps in the red pulp. No leukemic cells were seen in the white pulp (Figure 5A) and venous sinuses (Figure 5B). The white pulp was greatly enlarged (Figure 5A). Virus particles were rarely seen in the spleen. One of the 4 mice whose LF treatment was initiated at Day 0 and did not respond to the treatment had a large spleen, which was comparable in terms of its size and the

Table 1 — Schedule of Administration of LF and Control Medium to Mice Inoculated With the FVC*

		Days LF administered														
Group†	Dosage schedule	−14	−12	−10	−7	−5	−3	0	+1	+2	+3	+4	+7	+9	+11	+14
1	0							X		X		X	X	X	X	S
2	+1								X	X		X	X	X	X	S
3	+3										X	X	X	X	X	S
4	+7												X	X	X	S
5	+11														X	S
6	−14	X	X	X	X	X	X									S
7	−7				X	X	X									S
8	−3						X									S

*Mice were inoculated with FVC on Day 0. A total dosage of 200 μg LF was given to mice in the schedule as shown. Control mice received the same schedule of sterile pyrogen-free saline. The total dosage of 200 μg LF was administered in equal concentrations of LF. The days LF were given is marked by an x. When LF was started on Day 0, it was 4 hours after the administration of FVC. Mice were sacrificed (S) 14 days after receiving the FVC.

†Six mice per group.

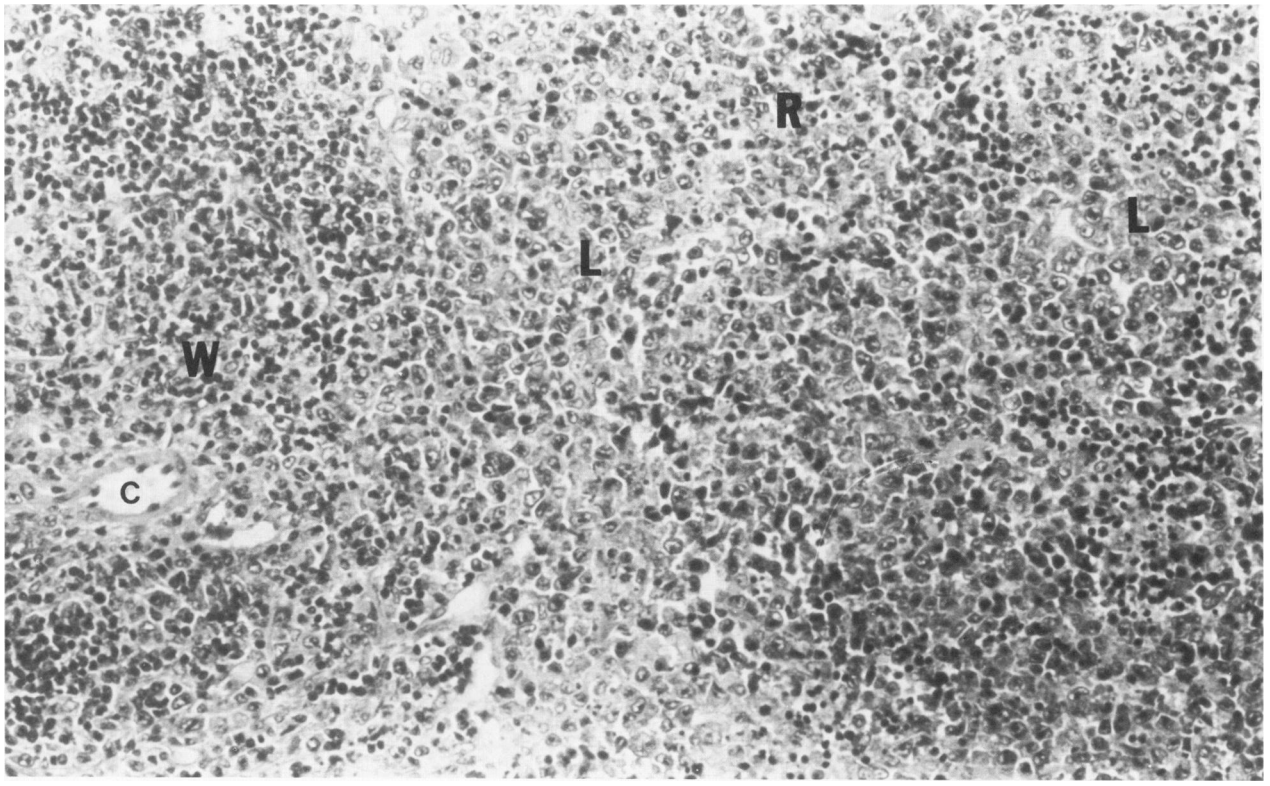

Figure 1 — Spleen of a mouse infected with FVC that did not receive LF treatment. Leukemic cells (*L*) occupied most of the red pulp (*R*) and invaded the white pulp (*W*), which became less discernible. *c*, central artery. (×340)

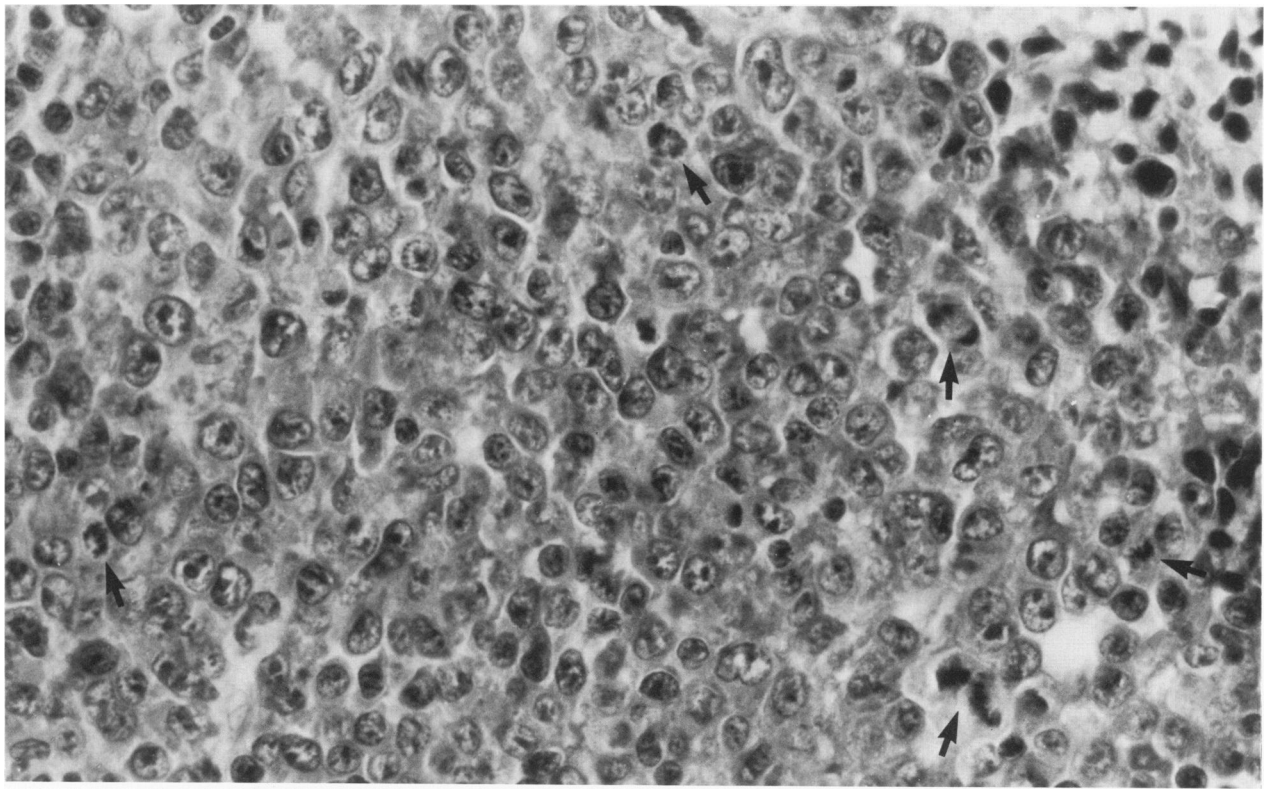

Figure 2 — Leukemic cells in the spleen of a mouse infected with FVC that did not receive LF treatment. Several cells (*arrows*) were in the process of cell division. (×700)

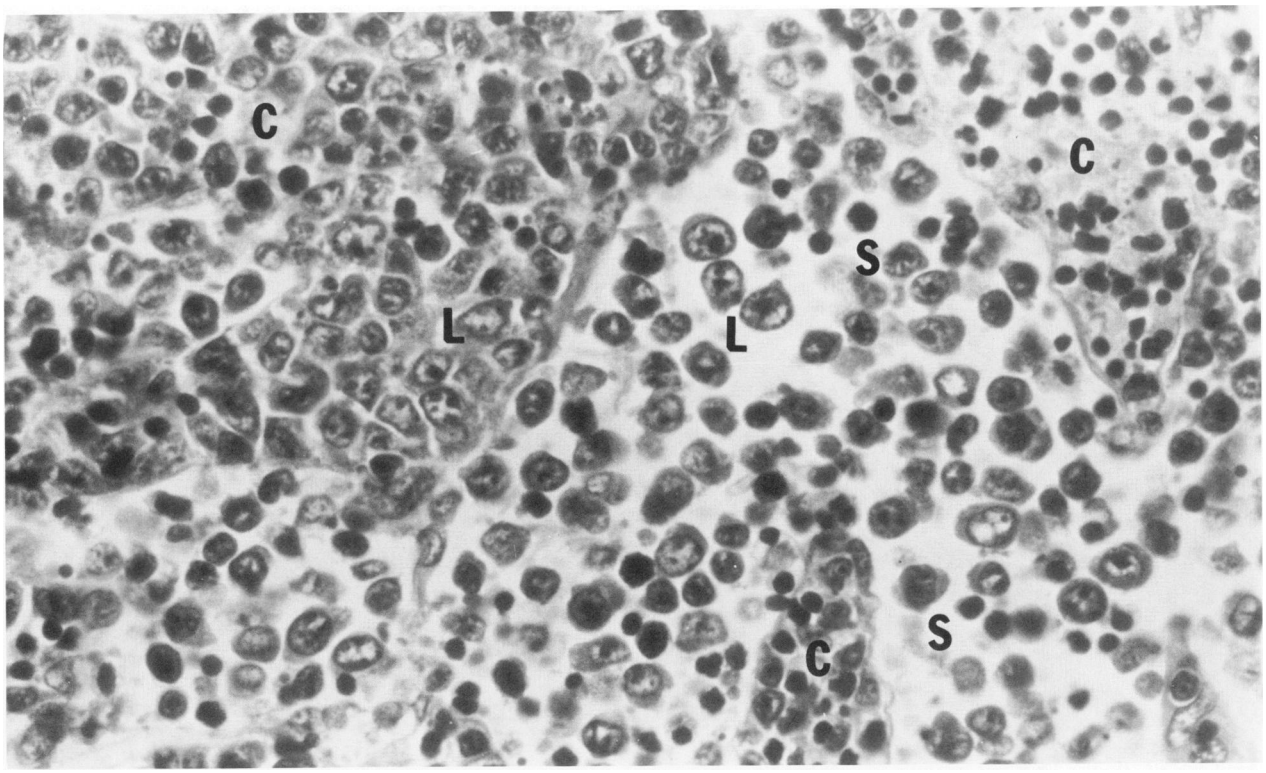

Figure 3 — Spleen of a mouse infected with FVC that did not receive LF treatment. Leukemic cells (*L*) not only filled most of the cordal space in the red pulp (*C*), but also appeared in venous sinuses (*S*). (×700)

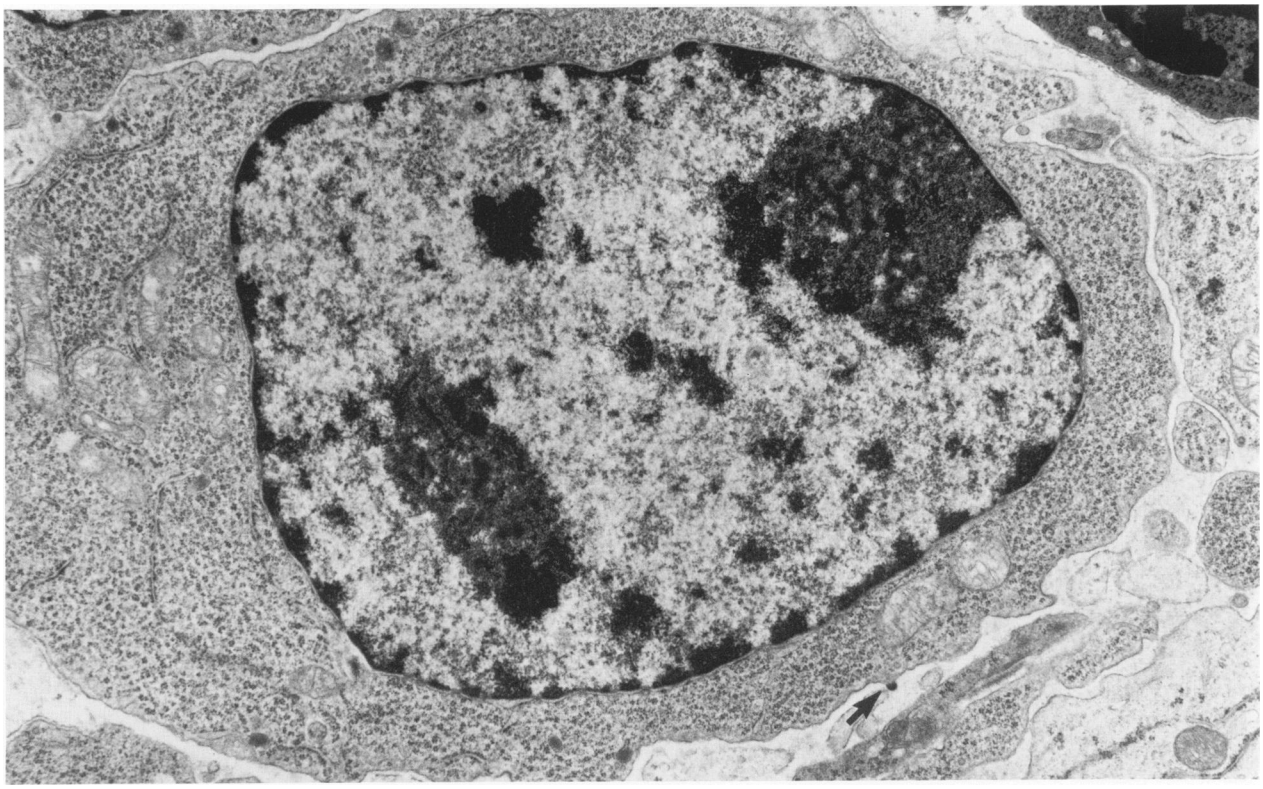

Figure 4 — A leukemic cell in the spleen of a mouse infected with FVC that did not receive LF treatment. Budding of a virus particle at the surface of the leukemic

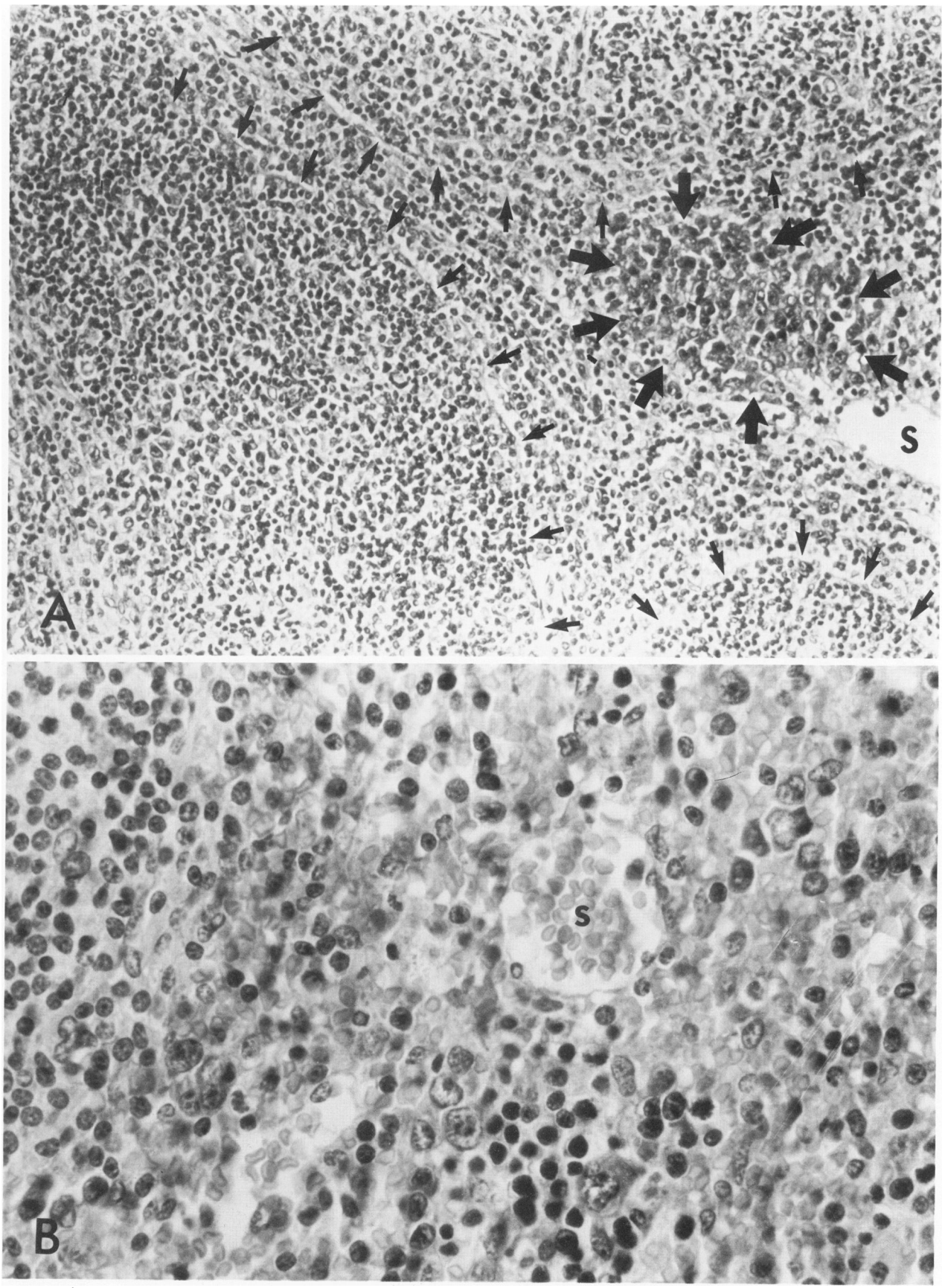

Figure 5 — Spleen of a mouse infected with FVC that received LF treatment at Day 1 after infection. Portions of the white pulp are outlined by *thin arrows*. A group of leukemic cells in the red pulp is indicated by *thick arrows*. No leukemic cells were present in the venous sinuses (*S*). (**A**, ×340; **B**, ×700)

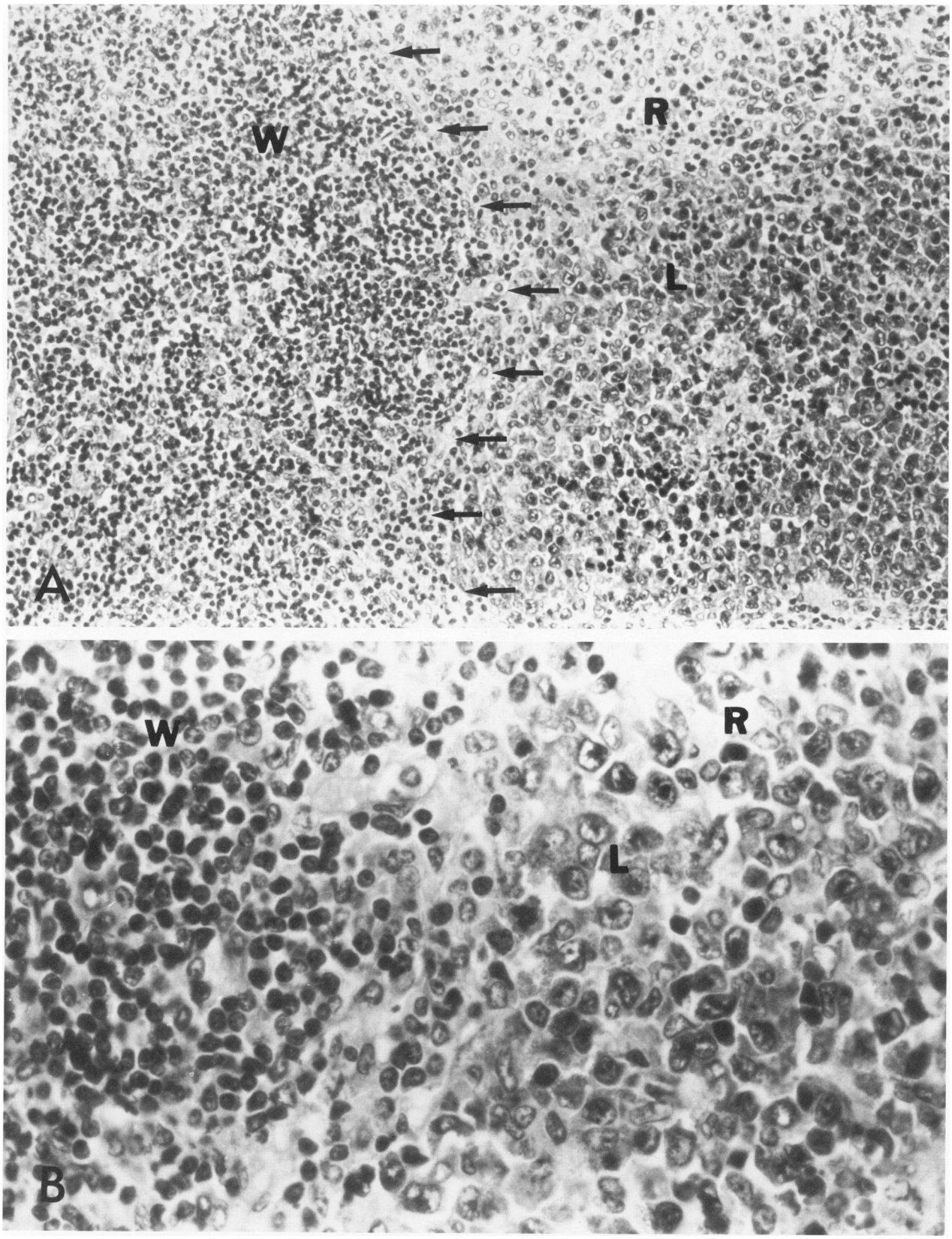

Figure 6— Spleen of a mouse infected with FVC that received LF treatment at Day 3 after infection. Leukemic cells (*L*) began to spread out in the red pulp and encroached upon the white pulp (*W*), outlined by *arrows*. The central portion of **A** is shown at a higher magnification in **B**. (**A**, ×340; **B**, ×700)

extent of leukemic infiltration to the spleen of mice infected with FVC and not given LF treatment.

In mice whose treatment was initiated at Day 3, leukemic cells began to spread out in the red pulp and encroached upon the white pulp. The white pulp was enlarged and clearly visible (Figures 6A and B). In mice whose treatment was initiated at Days 7 and 11 after viral infection, numerous leukemic cells were present in the red pulp. The white pulp was infiltrated by leukemic cells and became less discernible (Figure 7). The morphologic features of the spleen in mice whose treatment was initiated at Days 7 or 14 prior to viral infection were similar to those of the untreated groups as seen in Figures 1–3.

Discussion

The present study demonstrates that when FVC-infected mice responded to LF treatment,[1] ie, prolonged survival, the general architecture of the red pulp remained intact and the white pulp was enlarged but not infiltrated by leukemic cells. When infected mice did not respond to LF treatment, the red pulp as well as the white pulp was invaded by leukemic cells. Spleens were greatly enlarged, and the animals died within 3 months after viral infection.[1] The splenomegaly in mice that did not respond to LF treatment resulted mainly from an increased population of leukemic cells, whereas the splenomegaly in mice that responded to LF treatment was mostly due to an enlargement of the white pulp.

We do not have a precise method of measuring leukemic infiltration, but the estimation of the extent of leukemic infiltration in the spleen was based on the histologic observation of 1) the size of leukemic cell clumps in the splenic cords, 2) the diffusion of leukemic cells in the cordal space, 3) the appearance of leukemic cells in the sinuses, and 4) the appearance of leukemic cells in the white pulp.

The effectiveness of LF treatment in reducing the number of leukemic cells in the spleen appears to be dependent upon prompt initiation of LF treatment after viral infection. Treatments carried out 7 days before or after viral infection did not have any beneficial effects, and a great number of leukemic cells were observed in the spleen. The numbers of Friend virus particles detected in the spleen were proportional to the numbers of leukemic cells observed in the spleen.[1]

Leukemic cells were related morphologically to proerythroblasts.[8] Identification of the leukemic cells

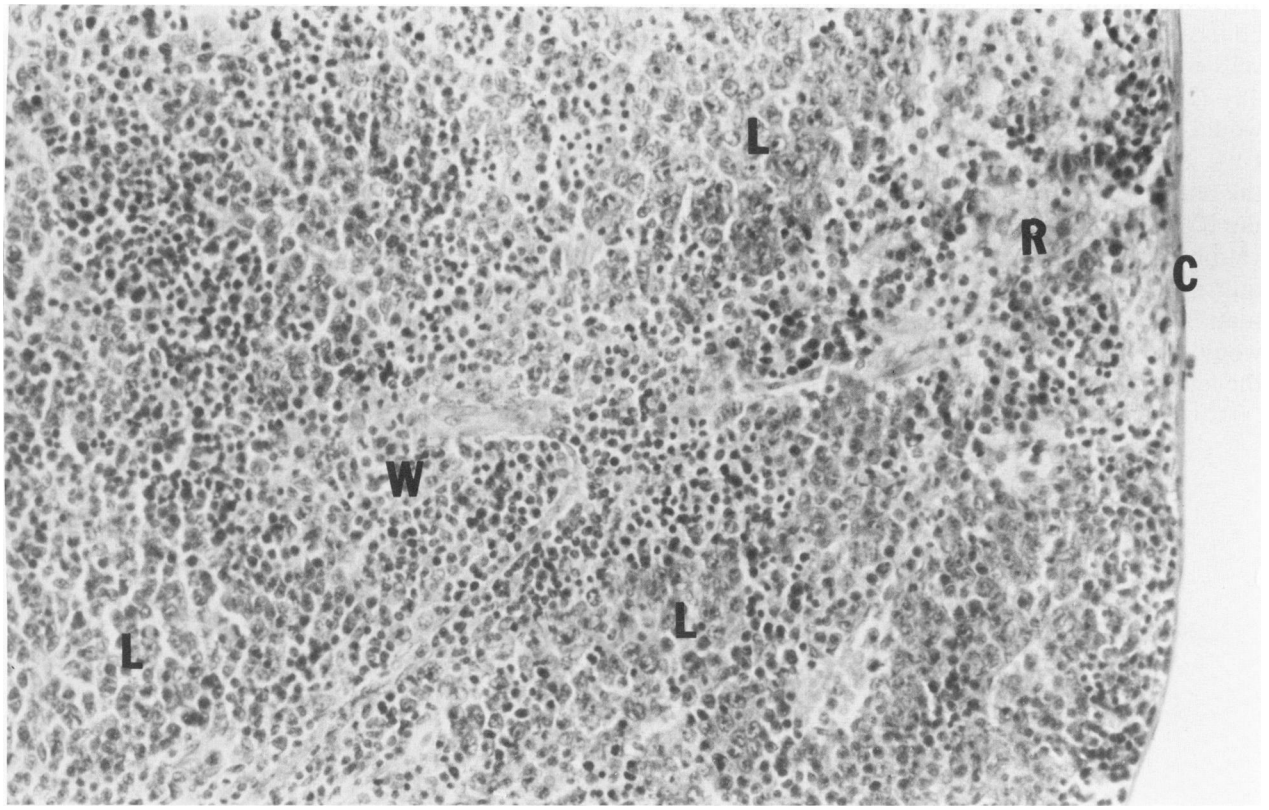

Figure 7 — Spleen of a mouse infected with FVC that received LF treatment at Day 7 after infection. Leukemic cells (*L*) occupied most of the red pulp (*R*) and invaded the white pulp (*W*), which became less discernible. *C*, splenic capsule. (×340)

were further supported by the presence of budding virus particles on the cell surface. Mice were not assessed for anti-human LF antibodies; so the potential role of such antibodies or their immune complexes is not known, and we did not check for the organ deposition of the administered LF. However, it is apparent from our data that LF has to be injected into mice early in comparison to the time the FVC is added in order for the protective effect of LF to be manifested *in vivo*. This is consistent with our studies *in vitro*, which have shown that macrophages taken from mice pretreated with FVC lack the capacity to respond to LF *in vitro* within a few days after the FVC inoculation.[3] On the basis of our studies with LF given to normal mice *in vivo* in which the LF decreased the cycling status of granulocyte-macrophage,[9,10] erythroid,[10] and multipotential[10] progenitor cells and the work of others demonstrating that virus infectivity is associated with the cycling of infected cells, we hypothesize that the long-term beneficial effects of the short-term administration of LF are due to a decrease in the cycling status of the hematopoietic progenitor cells, which is directly due to the ability of LF to decrease the production/release of growth factors from accessory cells which are needed for the proliferation of the progenitor cells. The effects of LF *in vivo* are reversible with time[9]; and when given as a single bolus dose *in vivo*, LF decreases the cycling status of cells within 6–12 hours, the effects being gone by 48–72 hours. A decrease in the cycling of the cells would make them less sensitive to infection by the FVC. LF has no direct effect *in vitro* on the SFFVs or the murine leukemia helper viruses which are part of the FVC (Lu, Oliff, Broxmeyer, unpublished observations). Since Friend leukemia development requires the permanent continuation of the transformation process,[11,12] arrest of the oncogenic viral effect would probably result in the rapid disappearance of the leukemic cells.[13]

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