cellulose column chromatography, and migration to the cathode during electrophoresis at pH 2.7, indicate their basic nature.

Arginine-rich proteins have also been reported in other types of neoplastic cells[2,3,7]. Until now their role within the cells has not been precisely established. It can be postulated that the basic proteins might be modulating factors in some process of intermediary metabolism in the cells.

On the other hand, it is known that these basic proteins are easily degraded into polypeptides in vitro by intracellular proteases and endopeptidases[8]. It can be supposed that there are proper substrates for the proteolytic enzymes. Those enzymes are easily released from lysosomes into the cytosol, and into the extracellular medium, by Ehrlich ascites tumour cells[9]. The existence of a well developed lysosomal system and the association of acid hydrolases with this kind of subcellular particle in Ehrlich ascites cells was established long ago[10]. Thus, a mechanism for the specific release of lysosomal contents, similar to the process studied in macrophages under the term 'exocytosis', may be suggested[11]. Low molecular weight arginine-rich peptides have been observed previously in the cytosol of some tumour cells, in extracellular spaces, and in the blood of tumour bearing rats[12,13].

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Compartmentalized growth of hemopoietic stem cells within mouse Friend leukemic spleens

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Summary. A dose of 4000 rads (r) to the central portion of mouse spleens followed by Friend erythroleukemic virus infection created independent compartments where hemopoietic stem cells exhibited distinct growth kinetics. Rather than suggesting autonomous proliferation, the stem cell kinetics were indicative of the control exercised by the local microenvironment upon stem cell growth within each compartment.

There is increasing evidence that self renewal of hemopoietic stem cells (HSC) depends upon local stimulation from specialized cells of the hemopoietic microenvironment. Although the nature of these cells is uncertain, their existence is suggested by a number of experiments in vivo[2-4] and in vitro[5]. These microenvironment cells have been called 'source' cells to emphasize their function as producers of a microdiffusing stimulus which promotes HSC renewal[6]. It is of interest now to see whether HSC renewal in mouse viral leukemia is also dependent upon stimulation from the microenvironment. As suggested elsewhere[6,7], Friend virus induces a leukemic response principally by increasing stimulus production by infected sources. Consequently, the population of HSC in leukemic spleens increases greatly above normal levels. However, when the spleen microenvironment is irradiated at doses $\geq$ 950 r the source cells (as any lethally irradiated cell) become resistant to virus infection[7,8] and support only a normal population of HSC.

We tested this hypothesis by irradiating with a single 4000 r dose only the central part of DBA/2j mouse spleens before injection of a large dose of Friend erythroleukemic virus. As detailed elsewhere[2] a number of normal, adult, male DBA/2j mice were positioned in the field of a 4 MV Varian linear accelerator. 2 (2 cm thick) lead blocks separated by a 5 mm gap were positioned over the mice to protect body and tail, with the exception of a 5 mm long central spleen portion, which received the full 4000 r dose. The extreme spleen portions (each about 5 mm long) were protected by the blocks and received $\leq$ 200 r as scatter. Since the spleens (average length = 15 mm) were not surgically exposed, positioning of the 5 mm gap was done by approximating their location in situ. This yielded only 3-5% of the mice with a centrally irradiated spleen. After irradiation all mice

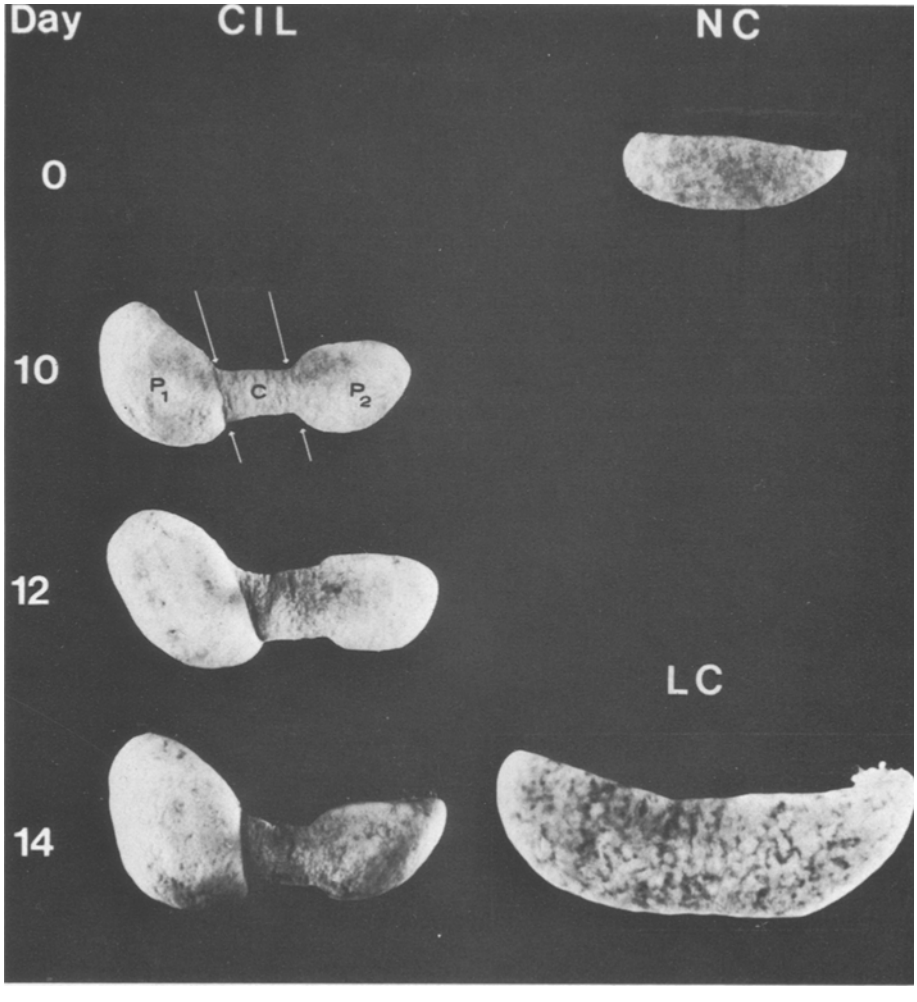
CFU content ($\pm$ SD) in separate portions of centrally irradiated and leukemic (CIL) spleens and in whole leukemic control (LC) spleens at various days after Friend virus injection

Day	CIL spleens			LC spleens	NC spleens
	P_1	C (4000 r)	P_2		
8	12,380 $\pm$ 3230	150 $\pm$ 60	8600 $\pm$ 2570	43,600 $\pm$ 6700	5400 $\pm$ 1580
10	15,100 $\pm$ 4060	890 $\pm$ 215	9980 $\pm$ 3200	48,320 $\pm$ 5920	5400 $\pm$ 1580
12	16,560 $\pm$ 4190	2350 $\pm$ 400	12,080 $\pm$ 2950	50,920 $\pm$ 8300	5400 $\pm$ 1580
14	17,940 $\pm$ 5280	2460 $\pm$ 520	13,830 $\pm$ 3800	60,560 $\pm$ 9870	5400 $\pm$ 1580

The P_1, C, and P_2 portions of CIL spleens are shown in the figure. NC spleens are from normal nonvirus injected control mice.

were injected with a maximal dose (0.2 ml) of a cell free Friend erythroleukemic virus as described elsewhere[9]. The spleens of these mice were identified as centrally irradiated and leukemic (CIL). As shown in the figure, a CIL spleen may be divided into 3 portions: P_1 and P_2, the protected extremes, and C, the central (4000 r) portion. As leukemic controls we used unirradiated mice injected with the same virus dose as above. Their spleens were labeled LC. As normal controls (NC) we used spleens from normal mice.

After virus infection the P_1 and P_2 portions of CIL spleens increased considerably in size with respect to portion C. This allometric growth became most evident from day 8 onward and contrasted with the isometric expansion of LC spleens. In Bouin-fixed spleens portion C was separated from P_1 and P_2 by sharp boundaries. The length of portion C (4.5 ± 0.5 mm) remained constant from day 8 through 14 (the last survival day) and corresponded to the 5 mm gap between the lead shields. In accord with previous experiments[2] irradiation created distinct growth compartments within the spleen. This aspect was further investigated by measuring the total CFU content in each CIL spleen portion. Starting with day 8, fresh CIL spleens were cut at the boundaries between P_1, C, and P_2 without discarding any tissue sections[2]. Cell aliquots from each portion were separately injected into 950 r DBA/2j test mice, and spleen colonies were counted 8 days after injection. From these counts the total CFU content (proportional to the HSC content) in each portion was calculated. The CFU numbers (table) showed that the HSC in the P_1 and P_2 portions grew as those in the LC spleens with a doubling time (t_D) of 11–14 days to a multiplicity much higher than that in normal (NC) spleens. The HSC in portion C, however, behave differently. Initially the HSC multiplied with a t_D of about 22 h up to day 10–11, when growth stopped at a level of about 2400 CFU, through day 14. This level, slightly higher than the 1800 CFU within the central ($^1/_3$) portion of a normal (NC) spleen, is 10-fold lower than the 24,000 CFU contained in the corresponding portion of a LC spleen during the same period. The hemopoietic cells growing in portion C were virus infected to the same extent as those in the P portions. When cell free virus extracts from equal volumes of each portion were injected into separate groups of normal mice, all mice developed typical and fatal Friend erythroleukemia. Considering that a 4000 r dose delivered to portion C destroyed all endogenous hemopoietic cells, the HSC seeding C had to come from those in the circulation. Their number increased steadily from 50 to about 800 CFU/cm^3 of blood on day 14. The possibility that seeding of portion C occurred by invasive growth of HSC from the P_1 and P_2 portions is not plausible because such an event would have gradually filled portion C, erasing the boundaries through convergent growth. On the contrary, the boundaries remained fixed, becoming sharper with time. As demonstrated by[2], the limited HSC growth in portion C cannot be explained by radiation damage to local vasculature, lack of space or nutrients, or due to toxic factors. Rather, it seems that a 4000 r microenvironment is blocked to stimulate no more than a normal HSC population even



Macroscopic appearance of centrally irradiated (4000 r) and Friend virus infected, leukemic spleens (CIL) with portions P_1 and P_2 protected from irradiation. The central portion, C, is 4.5 ± 0.5 mm long and is clearly visible between the boundaries corresponding to the edges of the lead shields. LC is a spleen from nonirradiated mice injected with the same virus dose as the CIL mice. NC is an average normal spleen 15 mm long. The sampling day is indicated from day 0, the time of irradiation and virus injection. The data in the table were collected from fresh spleens similar to those shown.

after exposure to a large virus dose and independent of whether the HSC per se are virus infected.

This study provides additional support to the suggestion that the microenvironmental stimulus promoting the HSC renewal can diffuse only over very short distances and certainly not throughout the C portion across the P_1-C-P_2 boundaries. (Otherwise, calculations show that portion C would have gradually disappeared in < 4 days after irradiation[2].) Finally, our results do not support the idea that Friend virus transforms infected HSC into autonomously growing cells. If so, the HSC within the C portion would have continued to grow with their initial t_D of 22 h to reach about 20,000 CFU by day 14. The HSC, however, slowed their renewal to a level of 2400 CFU, the stimulating capacity of a normal local microenvironment, in accordance with the original hypothesis. As a last remark, the interpretations of our results are valid only when the virus is injected after irradiation. Preliminary data (Matioli, unpublished) suggest that spleen microenvironments infected before irradiation may require a much higher radiation dose to reduce their stimulatory efficiency, perhaps because unirradiated microenvironmental sources become hyperactive rapidly after virus infection.

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Skeletin immunoreactivity in peripheral nerves

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Summary. By use of monospecific antibodies against the cow heart intermediate filament protein, skeletin, an antigenic relationship between skeletin and neurofilament protein of peripheral nerves is demonstrated. Crossreactivity is also demonstrated in the filament-containing Schwann cells. The results are consistent with the existence of several subclasses of related intermediate-sized filament proteins.

Neurofilaments occur in most nerve cells and comprise a major structural component of the myelinated axons of most species[3,4]. Neurofilaments seem to be the neural variant of a filament class which is present in all types of eukaryotic cells. Until recently, the identification of these filaments has been restricted to electron microscopic studies in which they have been characterized as smooth-surfaced, fairly rigid filamentous organelles of indeterminable length and with a diameter of 8–10 nm[5,6]. For this reason, the filaments of nonneural tissues have often been called '10 nm filaments' or 'intermediate filaments', as they have an intermediate diameter as compared with actin and myosin filament[5].

The purification of the neurofilament protein[7] and the glial filament protein (GFA)[8], made possible the production of antisera and the application of immunomicroscopic methods to the field of neurofilament research. Such investigations would have been expected to elucidate the immunological relatedness of neurofilaments, glial filaments and intermediate filaments from other cells. However, the results from such immunomicroscopic studies have been confusing in the sense that the question of cross-immunoreactivity has been answered in different ways by different authors. Evidence of both cross-reactivity[9–11] and nonreactivity[12–14] have been presented[15].

Recently, we have reported a method for preparing antibodies to intermediate filament protein (skeletin) from the cow heart conduction system, a cell system of myogenic origin buth with primarily nerve-like properties[16]. We have now adopted the indirect immunofluorescence method in order to demonstrate the immunoreactivity of heart skeletin in peripheral nerves. The results suggest that skeletin filaments and neurofilaments share antigenic determinants.

Experimental. The study was performed in the lateral cutaneous nerve of the thigh (n cutaneus femoris lateralis) of the cow, obtained within 5 h after death. Specimens were frozen in Freon-12 chilled with liquid nitrogen. Longitudinal and cross sections were cut in a cryostat and mounted on glass slides. The mounted sections were fixed in cold absolute acetone and air dried. Incubations were carried out in antiskeletin prepared as previously described[16] and diluted 1:50 in phosphate buffered saline (PBS) (1 h, +37 °C, moist chamber). After repeated washes in PBS, sections were incubated in FITC-conjugated goat antirabbit globulin (GARG) under the same conditions as for antiskeletin, washed in PBS and mounted in PBS-glycerol.

Control sections were treated in a similar way but with absorbed or nonimmune sera or with GARG alone. Sections were viewed in a Leitz Orthoplane Photomicroscope with epifluorescent optics, or in a Leitz Dialux-20 Photomicroscope equipped with interference contrast accessoires.

Results. In crosscut nerves a characteristic starry sky appearence was observed, consistent with the staining of myelinated axons. Surrounding myelin was not stained, but a distinct staining did occur in the Schwann cell cytoplasm. Endoneurial and perineurial connective tissue were negatively stained (figure 1), while small vessels showed specific fluorescence of the smooth muscle layer. The findings were confirmed by correlation with interference contrast microscopy of identical areas (figure 3) and sections stained with Gomori trichrome (figure 4). In longitudinal sections, the axons appeared as slender fluorescent ribbons while surrounding areas were dark. Control sections were all dark except for a faint nonspecific staining of myelin sheaths (figure 2).

Discussion. The demonstration of skeletin immunoreactivi-