Proc. Natl. Acad. Sci. USA
Vol. 73, No. 10, pp. 3710–3713, October 1976
Microbiology

C-type virus particle formation in erythroblastic islands in spleens of C3Hf mice injected with erythropoietin

(mouse leukemia virus development/erythropoiesis)

DOROTHY FELDMAN

Department of Experimental Pathology, Hoffmann-La Roche Inc., Nutley, New Jersey 07110; and Cancer Research Unit, Veterans Administration Hospital, Bronx, New York 10468

Communicated by Ludwik Gross, July 12, 1976

ABSTRACT **Previous studies suggested a possible association between C-type virus particle formation and erythropoiesis. In this experiment, normal C3Hf mice were injected with erythropoietin in order to increase the rate of erythropoiesis. Sections of spleen from these animals revealed extensive areas of erythroblasts. C-type particles, budding from erythroblasts, were often observed in such areas of spleen from every mouse injected with erythropoietin.**

In contrast, in sections of spleen from control mice, zones of erythropoiesis were limited, and only a few scattered C-type virus particles were observed in some of the animals examined.

An association between C-type virus particle formation and erythropoiesis was initially suggested when C-type particles were observed budding from cells in erythropoietic foci in C3Hf mouse embryonic spleen, liver, and bone marrow (1). Subsequent findings presented in a recent report (2) lent support to this original assumption. As erythropoietic zones in 1½- to 18-day-old C3Hf mice became less prominent, fewer C-type particles were observed. Also, examination of the site of earliest erythropoiesis, the yolk sac blood islands, revealed the presence of C-type particles.

The experiment described here was devised to further test the relationship between C-type particle formation and erythropoiesis. To increase the rate of erythropoiesis, several groups of C3Hf mice were injected with erythropoietin. This is a report of the initial observations of C-type particle formation in erythroblastic islands in the spleens of these mice.

MATERIALS AND METHODS

Experiment I. Twelve C3Hf mice, 2 to 3 months old, from the colony of pedigreed mice of this inbred line, bred by brother to sister mating in the Bronx V.A. Hospital, Cancer Research Unit, were used in this experiment. Each of six mice were injected subcutaneously with 12 units of erythropoietin* dissolved in 0.9% NaCl. Six control mice were injected subcutaneously with 0.9% NaCl only. The animals were divided into three groups, consisting of two experimental and two control animals per group. Group I was injected on day one and was sacrificed on day two. Group II was injected on day one and on day two, and was sacrificed on day three. Group III was injected on day one and on day two and was sacrificed on day four. From day one to the day of sacrifice, blood was drawn daily from the tail of each animal for hematocrit and hemoglobin determinations. The spleen was removed from each animal for study.

Experiment II. Experiment II was identical to experiment I except that the mice were not bled for hemoglobin and hematocrit determinations.

* Obtained from Connaught Labs., Willowdale, Ontario, Canada; grade step III, freeze-dried powder prepared from plasma of sheep made anemic by treatment with phenylhydrazine.

Experiment III. Experiment III was identical to experiment II except that an additional two experimental mice and two control mice (group IV) were injected on day one and on day two and were sacrificed on day six.

Tissues for electron microscopy were fixed in 3% phosphate-buffered glutaraldehyde, rinsed in buffer, and post-fixed in 1% phosphate-buffered osmic acid. Specimens were then processed and consecutive thick and thin sections were prepared as previously described (2, 3). Sections were examined in a Philips 300 electron microscope at 60 kV.

RESULTS

Spleens of mice injected with erythropoietin

Spleens from mice injected with erythropoietin contained extensive erythropoietic foci. C-type virus particles could be located in erythroblastic islands in spleen of every mouse from all the groups given erythropoietin in experiments I, II, and III. Sections covering ¼ to ⅓ of a 300 mesh grid contained approximately 10 to 25 particles per grid (Table 1). Particles budding from erythroblasts and immature particles were observed; mature particles were rarely found. Figs. 1–3 are electron micrographs of groups of erythroblasts. Figs. 1a and 2a are enlargements of areas indicated by arrows in Figs. 1 and 2, and demonstrate the presence of C-type virus particles budding from erythroblasts. Higher magnifications (Fig. 3a–c) of areas a, b, and c in Fig. 3 also illustrate budding particles. In Fig. 4, two particles (arrows) are shown budding from the same erythroblast. An immature C-type particle adjacent to an erythroblast appears in Fig. 5 (arrow).

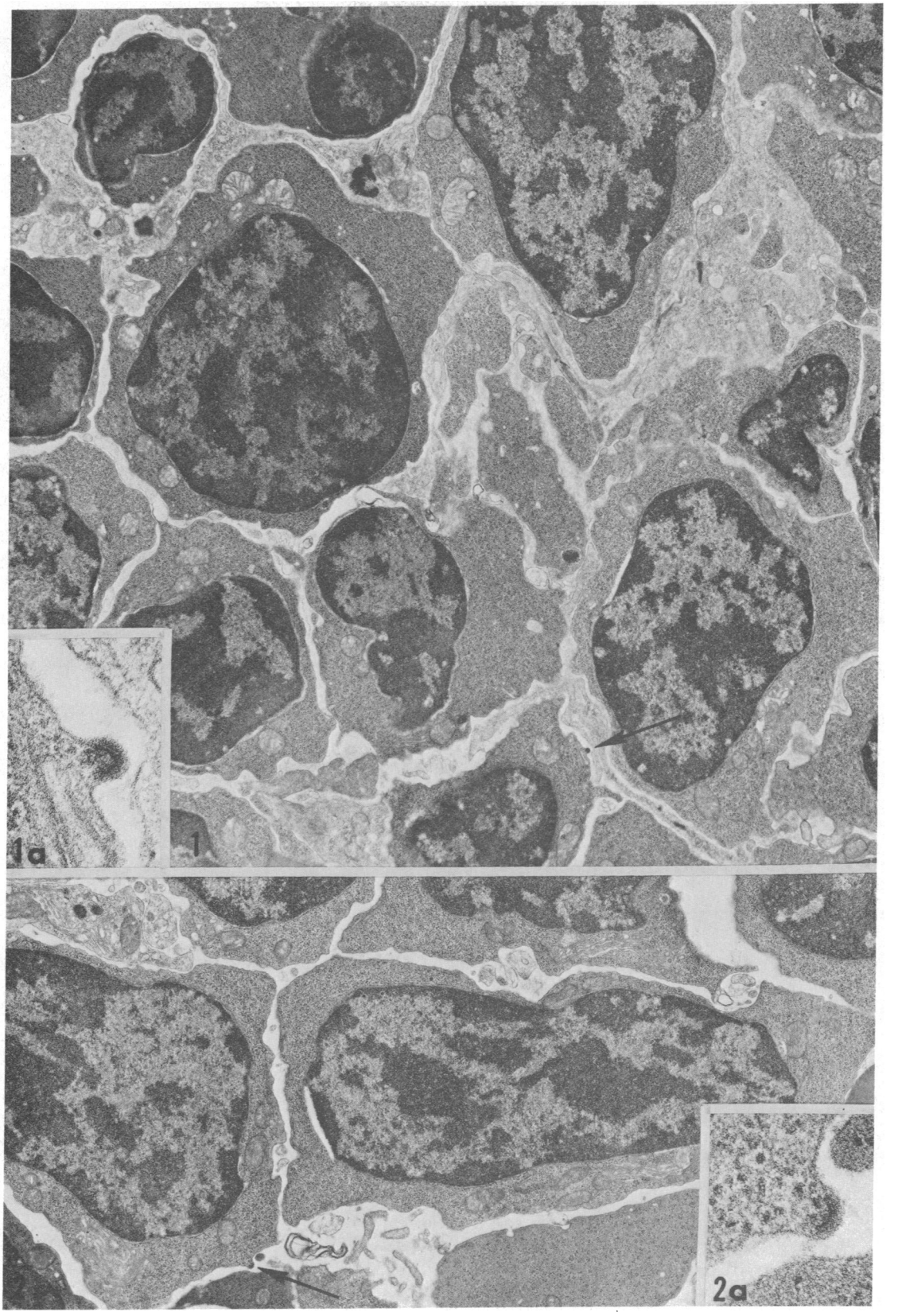
In sections of spleens from mice sacrificed on day six, erythroblastic islands were not observed; a single C-type particle was found in spleen from one of these animals.

Spleens of control mice

In sections of spleens from control mice of experiments II and III, erythroblasts were either sparse or not located at all. However, in some sections of spleen from animals in experiment I, in which the mice were bled, a few small clusters of erythroblasts could be found. In spleens of control mice from experiments I, II, and III, particles were observed with less frequency and were considerably fewer in number than in mice injected with erythropoietin (Table 1). Some scattered particles, approximately one to five per grid, were observed in spleens from four of six mice examined in experiment I, in two of six mice in experiment II, and in two of six mice in experiment III. C-type particles were not observed in spleens of the two mice sacrificed on day six.

DISCUSSION

Results of this experiment corroborate earlier findings (1, 2) of the association between erythropoiesis and C-type virus particle



FIGS. 1 AND 2. Spleen from mouse sacrificed 2 days after second injection of erythropoietin which illustrates zones of erythroblasts. The magnification in Fig. 1 is ×17,785; in Fig. 2 ×20,520.

FIGS. 1a AND 2a. Higher magnifications of areas marked by arrows in Figs. 1 and 2 show C-type virus particles budding from erythroblasts. The magnification in Fig. 1a is ×93,290; in Fig. 2a ×100,700.

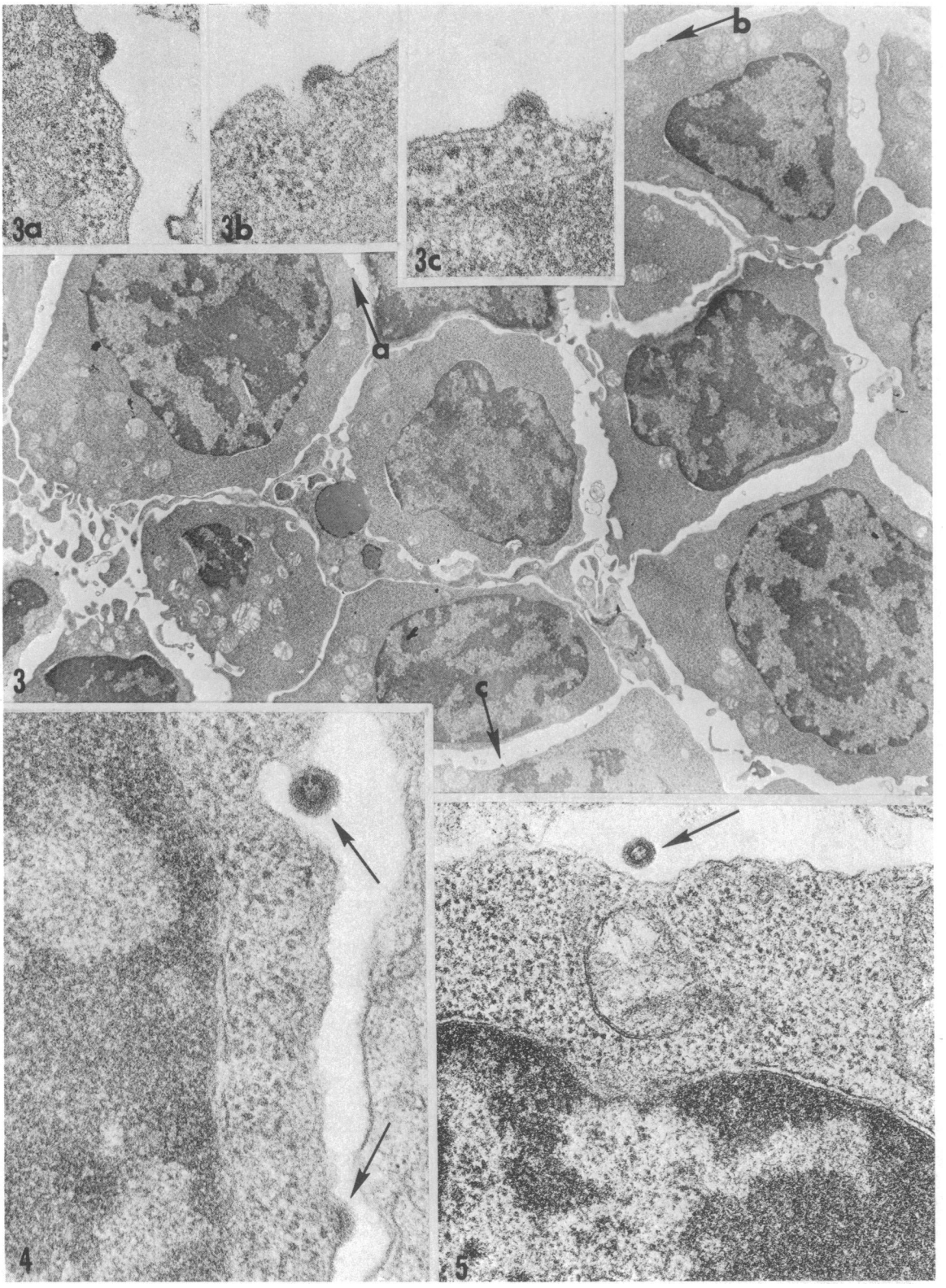


FIG. 3. Area of erythroblasts from spleen of mouse sacrificed 1 day after second injection of erythropoietin. Fig. 3a, b, and c of areas, a, b, and c, respectively, demonstrate, at higher magnifications, C-type virus particles budding from erythroblasts. The magnification in Fig. 3 is ×12,010; in Fig. 3a ×67,615; in Fig. 3b ×85,540; and Fig. 3c ×85,540.

FIG. 4. Part of an erythroblast from spleen of mouse sacrificed 1 day after second injection of erythropoietin. Two particles (arrows) are shown budding from the cell. ×96,460.

FIG. 5. Part of an erythroblast from spleen of mouse sacrificed 1 day after first injection of erythropoietin. An immature C-type virus particle (arrow) is shown adjacent to the cell. ×64,610.

Table 1. Comparison of C-type virus particles in spleens of control and erythropoietin-injected C3Hf mice

	Erythropoietin-injected	Control
No. mice positive / No. mice examined	18*† / 18	4* 4† / 6, 12
No. particles‡	10–25	1–5

* Experiment I.
† Experiments II and III.
‡ Per grid examined: sections covering ¼ to ⅓ of a 300 mesh grid.

formation in normal C3Hf mice from this colony. After injection of erythropoietin, the rate of erythropoiesis was increased which results in the formation of erythroblastic islands in the spleen; C-type virus particles were found budding from erythroblasts in the spleen of every mouse injected with erythropoietin. As compared to erythropoietin-injected mice, particles were observed in fewer animals and in smaller numbers in the spleens of control mice, especially in experiments II and III. It thus appears that erythropoiesis stimulates the production of C-type virus particles from erythroid cells.

Bleeding the animals as shown for experiment I resulted in a loss of blood which was evidenced by a continuous daily reduction in the hematocrit and hemoglobin values in both experimental and control mice. Prior to the first injection, the hematocrits of the mice in group III ranged from 48 to 51% and the hemoglobins ranged from 15 to 15.6 g. Before the mice were sacrificed, after being bled four times, the hematocrits of the mice in this group ranged from 37 to 40% and the hemoglobins from 12 to 13.1 g. Erythropoiesis in the control groups appeared to be enhanced by bleeding the mice. Erythroblasts and C-type particles were found in the spleens of more control mice of experiment I than experiments II and III.

Other recent experiments on the effect of erythropoietin on virus-infected erythroid cells include a study which demonstrated that virus-infected erythroid precursor cells from mice with anemia induced by a slow-acting strain of Rauscher leukemia virus responded to erythropoietin administration by differentiating into erythroid cells (4). In another study (5), hematopoietic cells from mice infected with Friend virus were shown to be capable of erythroid differentiation *in vitro* independent of erythropoietin. However, in this experiment, erythropoietin-induced cellular differentiation in normal nonleukemic C3Hf mice resulted in the formation of C-type virus particles from erythroid cells which suggests a possible relationship between the mechanisms involved in erythroid cellular differentiation and those responsible for the formation of C-type virus particles.

Normal C3Hf mice are known to contain latent and potentially leukemogenic viruses which are usually non-pathogenic under normal conditions (6, 7). However, various endogenous or exogenous factors such as certain hormones, carcinogenic chemicals, or ionizing radiation may activate these latent viruses. Fractionated total body x-ray irradiation of C3Hf mice results in a significant increase in quantity and organ distribution of C-type virus particles (8) and in the development of of radiation-induced leukemia in the majority of these mice (9). A filterable and transmissible leukemogenic virus could be recovered from their tissues (10). The C-type virus particles observed in this experiment may represent such a latent virus. Experiments are currently in progress to investigate the possible pathogenic potential of the virus particles formed in the spleens of C3Hf mice following injection of erythropoietin.

1. Feldman, D. G., Dreyfuss, Y. & Gross, L. (1967) "Electron microscopic study of the mouse leukemia virus (Gross) in organs of mouse embryos from virus-injected and normal C3Hf parents," *Cancer Res.* **27**, 1792–1804.
2. Feldman, D., Gross, L. & Swarm, R. L. (1976) "Electron microscopic study of virus particles in yolk sac and placenta and in hematopoietic organs of newborn C3Hf mice," *Cancer Res.* **36**, 1814–1820.
3. Feldman, D. (1975) "An electron microscopic study of virus particles in rhesus monkey placenta," *Proc. Natl. Acad. Sci. USA* **72**, 118–121.
4. Weitz-Hamburger, A., Fredrickson, T. N., LoBue, J., Hardy, W. D. Jr., Camiscoli, J. F., Ferdinand, P., Gallicchio, V. & Gordon, A. S. (1975) "Stimulation of erythropoietic differentiation in BALB/c mice infected with Rauscher leukemia virus," *J. Natl. Cancer Inst.* **55**, 1171–1175.
5. Liao, S.-K. & Axelrad, A. A. (1975) "erythropoietin-independent erythroid colony formation *in vitro* by hemopoietic cells of mice infected with Friend virus," *Int. J. Cancer* **15**, 467–482.
6. Gross, L. (1954) "Is leukemia caused by a transmissible virus? A working hypothesis," *Blood* **9**, 557–573.
7. Gross, L. (1974) "Facts and theories on viruses causing cancer and leukemia," *Proc. Natl. Acad. Sci. USA* **71**, 2013–2017.
8. Gross, L. & Feldman, D. G. (1968) "Electron microscopic studies of radiation-induced leukemia in mice: Virus release following total-body x-ray irradiation," *Cancer Res.* **28**, 1677–1685.
9. Gross, L., Roswit, B., Mada, E. R., Dreyfuss, Y. & Moore, L. A. (1959) "Studies on radiation-induced leukemia in mice," *Cancer Res.* **19**, 316–320.
10. Gross, L. (1958) "Attempt to recover filterable agent from x-ray-induced leukemia," *Acta Haematol.* **19**, 353–361.