

## Helper virus is not required for *in vitro* erythroid transformation of hematopoietic cells by Friend virus

(Friend polycythemia virus/spleen focus-forming virus/murine leukemia virus/kinetics/helper-involvement)

W. DAVID HANKINS\* AND S. B. KRANTZ†

\*Departments of Medicine and Biochemistry, Vanderbilt University Medical School, Nashville, Tennessee 37232; and †Veterans Administration Medical Center, Division of Hematology, Nashville, Tennessee 37203

Communicated by Sidney P. Colowick, June 2, 1980

**ABSTRACT** The Friend polycythemia virus complex (FVP), consisting of the replication-defective spleen focus-forming virus (SFFV) and a helper Friend murine leukemia virus (MuLV-F), produces erythroleukemia within 2-3 weeks *in vivo*. We have recently reported *in vitro* transformation of bone marrow cells by FVP, producing clusters of erythroid colonies (erythroid bursts) 4-6 days after infection. In contrast to uninfected bone marrow cells, FVP-treated cells proliferated and differentiated (synthesized hemoglobin) in the absence of added erythropoietin, the physiologic regulator of erythropoiesis. The relative roles of helper murine leukemia virus (MuLV) and SFFV in the *in vitro* erythroid transformation have now been examined. Pseudotype studies and the finding that cloned MuLV-F (free of SFFV) did not induce burst formation indicated that SFFV was essential for this *in vitro* effect of FVP. Because SFFV could not be obtained free of helper MuLV, we assessed the requirement of MuLV in the transformation by kinetic analyses of helper-deficient and helper-excess FVP preparations. Whereas helper-excess FVP gave single-hit kinetics both *in vivo* and *in vitro*, the helper-deficient FVP followed multiple-hit kinetics when titrated for spleen focus formation *in vivo*. Addition of MuLV-F to helper-deficient FVP prior to injection resulted in a marked enhancement of spleen focus formation and a conversion from multiple-hit to single-hit kinetics. In contrast, titration of this same preparation for erythroid burst transformation *in vitro* yielded single-hit kinetics, and the addition of helper MuLV-F had no effect. The time course of burst development was similar with or without added MuLV-F. Unlike burst transformation, SFFV production by these infected cultures followed multiple-hit kinetics. Addition of MuLV-F at the time of infection led to an enhancement of SFFV production and conversion of the titration curve from multiple-hit to single-hit. These data are consistent with the idea that SFFV is competent for erythroid transformation *in vitro*, but requires helper MuLV for its replication.

Two manifestations of the early erythroid response to the Friend polycythemia virus complex (FVP) (1, 2) are the formation of macroscopic spleen foci (3) 9 days after infection of mice and the progressive appearance of cells in the spleen and marrow that differentiate into erythroblasts *in vitro* in the absence of added erythropoietin (4-7). The agent responsible for these early erythroid responses is thought to be the spleen focus-forming virus (SFFV), which is replication-defective and requires the presence of a helper murine leukemia virus (MuLV) for production of progeny SFFV (8-10). From studies in which SFFV was titrated in genetically resistant mice (11), it is evident that the formation of macroscopic spleen foci *in vivo* is a multihit phenomenon requiring both SFFV and

MuLV. Experiments involving the infusion of FVP-infected cells into mice indicate that virus replication, with secondary infection and transformation of other hematopoietic cells, occurs in the process of focus formation and that the MuLV requirement can be explained by its role in the production of SFFV (12, 13). However, these requirements *in vivo* do not provide information on the relation of the MuLV to the primary transformation process.

We have demonstrated that the early erythroid transformation of murine marrow or spleen cells by FVP can be produced entirely *in vitro* (14, 15). In this system, incubation of hematopoietic cells with FVP preparations leads to the production within 5 days of multiple clusters of erythroid colonies that have been described as bursts. A constant association between the production of erythroid bursts *in vitro* and the production of spleen foci *in vivo* by infectious plasma has led to the conclusion that SFFV is necessary for the erythroid transformation *in vitro* (14, 16). Two observations have indicated that helper MuLV is not directly responsible for this *in vitro* transformation. First, several preparations that contained only MuLV did not produce erythroid bursts *in vitro*. Second, when Moloney MuLV (MuLV-M), which has no erythroid-inducing activity *in vivo* or *in vitro*, was used to rescue SFFV from nonproducer cells, the SFFV (MuLV-M) pseudotypes produced erythroid bursts very efficiently (14-16). Whereas these studies have shown that MuLV alone cannot induce erythroid burst transformation *in vitro*, they have not delineated the role, or lack of a role, that MuLV might have in conjunction with SFFV for erythroid transformation. In this study, we have performed kinetic experiments using helper-deficient and helper-excess preparations of FVP in order to assess the relation of MuLV to the production of erythroid bursts *in vitro* by SFFV. Our experiments indicate that helper MuLV is not required for the erythroid transformation *in vitro*.

### METHODS

**Virus Preparations and Assays.** The NB-tropic helper-excess FVP contained  $10^7$  XC (17) plaque-forming units (PFU) per ml and  $2 \times 10^5$  focus-forming units per ml in the 9-day spleen focus-forming assay (3). This preparation was originally obtained in 1967 from Robert Holden at the National Institutes of Health and has been maintained by serial passage of infectious plasma in BALB/c mice since that time. The NB-tropic helper-deficient preparation was harvested from a cell

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MuLV, murine leukemia virus; MuLV-F, Friend murine leukemia virus; MuLV-M, Moloney murine leukemia virus; FVP, Friend polycythemia virus complex; SFFV, spleen focus-forming virus; PFU, plaque-forming units; MSV, murine sarcoma virus.

line of Friend virus-infected NIH/3T3 cells described by Bernstein *et al.* (18). This preparation contained  $3 \times 10^8$  PFU and  $10^3$  focus-forming units per ml. XC assays were carried out on SC-1 cells as described (17). Spleen focus-forming assays were performed in BALB/c mice as described by Axelrod and Steeves (3). The MuLV-F helper was obtained by two cycles of endpoint dilution of the helper-excess FVP. This MuLV-F stock was used to infect SC-1 cells, and the supernatant fluid was harvested when the cells reached confluency. The particular preparation used herein contained  $10^6$  PFU/ml. No spleen foci were observed 2 months after inoculation of BALB/c mice with 0.2 ml of the MuLV-F preparation.

**Production of Erythroid Bursts by FVP *in Vitro*.** For virus adsorption, marrow cells were prepared from mice that had been erythropoietically stimulated by phenylhydrazine treatment (14, 15). Bone marrow suspensions of  $4 \times 10^6$  cells were incubated with 300  $\mu$ l of various dilutions of the virus preparations. After 2 hr at 40°C, the cells were plated in methylcellulose cultures and scored for the production of erythroid bursts at 5 days as described (15).

**Cocultivation of FVP-Infected Hematopoietic Cells with SC-1 Fibroblasts.** Preliminary results indicated that SFFV production by helper-deficient FVP-infected cells was low. To increase the sensitivity of SFFV detection, virus-infected hematopoietic cells were cocultivated with SC-1 fibroblasts. SC-1 cells ( $10^5$ ) were seeded in 60-mm culture dishes (Falcon). Cells were pooled from each hematopoietic culture that had been infected 6 days earlier with varying dilutions of helper-deficient FVP in the presence or absence of added helper MuLV-F. The hematopoietic cells were washed once with Hanks' balanced salt solution, pelleted, and resuspended in 8 ml of growth medium [Eagle's minimal medium containing 10% (vol/vol) fetal calf serum (Reheis, Kankakee, IL) with 2  $\mu$ g of Polybrene (Aldrich) per ml]. This suspension (4 ml) was added to 60-mm Falcon dishes (Falcon) in which  $10^5$  SC-1 cells had been seeded 24 hr earlier. Following 2 days of cocultivation, the medium and hematopoietic cells were discarded and the SC-1 cells were passaged one time. When the SC-1 cells reached confluency, the supernatants of replicate cultures were pooled and clarified by centrifugation (1400  $\times$  g for 5 min). The supernatants were diluted and assayed for SFFV after addition of excess MuLV-F as helper ( $10^4$  PFU/ml).

## RESULTS

**Titration of Helper-Excess and Helper-Deficient FVP *in Vivo*.** Serial dilutions of a helper-excess preparation of infectious plasma were injected into BALB/c mice. When 0.2 ml of a 1:10,000 dilution was injected, an average of seven foci per spleen was observed. As the preparation was further diluted, the number of foci per spleen decreased roughly in proportion to the dilution. In Fig. 1A, these data are plotted in the manner proposed by Hartley and Rowe (19) for murine sarcoma virus (MSV) titrations. With this method, a single-hit pattern is indicated by a straight line parallel to the x axis, while a multihit curve falls toward the x axis with the square of the dilution factor. The titration pattern observed in Fig. 1A with the helper-excess preparation is representative of single-hit kinetics. As expected, addition of MuLV-F ( $10^4$ /ml) did not alter the number of foci per spleen or the titration pattern. We next titrated another FVP preparation, which was thought to be deficient in helper MuLV (Fig. 1B). In this case, the titration curve fell toward the x axis as increasing dilutions were injected. When MuLV-F was added to the helper-deficient FVP preparation prior to its injection into the mice, two changes were noted. First, there was a significant enhancement of the number of foci per spleen, particularly at the higher dilutions. Second,

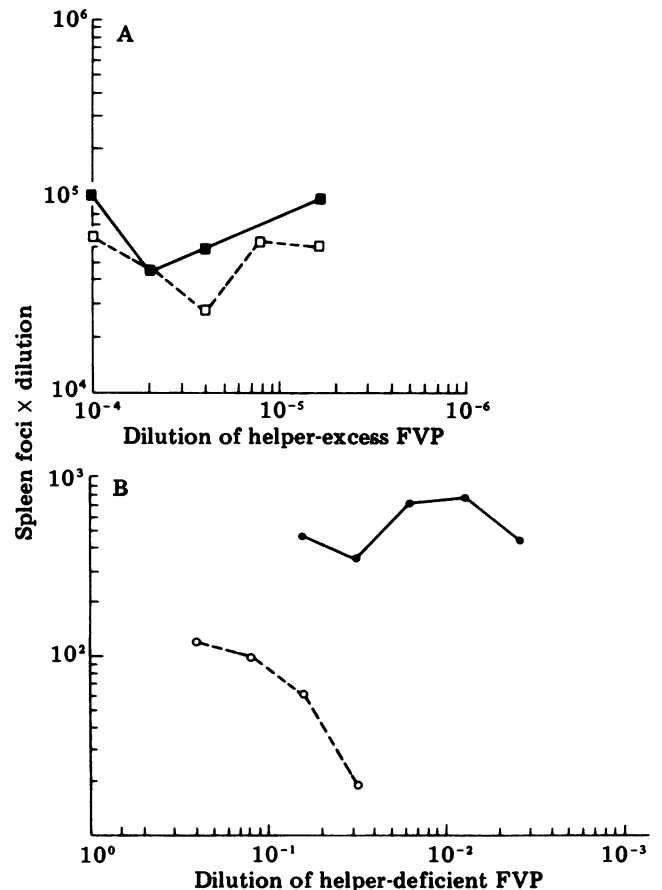


FIG. 1. Kinetics of spleen focus formation by FVP. (A) Helper-excess FVP ( $10^7$  PFU/ml) was appropriately diluted in Hanks' balanced salt solution and divided into two parts. To one part (●) sufficient helper MuLV-F was added to give  $10^4$  PFU/ml. No helper was added to the second half (□). Both preparations were injected intravenously into 4-6-week-old BALB/c mice (0.2 ml/mouse). Each point represents the mean of seven mice per group. Splenic foci were scored at 9 days. (B) Helper-deficient FVP ( $10^3$  PFU/ml) was similarly assayed with (●) or without (○) added helper MuLV-F.

the titration pattern was converted from a multihit to a single-hit curve. Both these findings indicate that the virus preparation was helper-deficient.

**Titration of Helper-Excess and Helper-Deficient FVP *in Vitro*.** Bone marrow cell suspensions were incubated with varying dilutions of the helper-excess (Fig. 2A) or the helper-deficient (Fig. 2B) FVP. Parallel experiments also were carried out in which MuLV-F ( $10^4$ /ml) was added to these preparations prior to their incubation with bone marrow cells. As indicated by the horizontal titration curves in Fig. 2A and B, the number of erythroid bursts produced *in vitro* varied directly with the concentration of each of these virus preparations. The addition of MuLV-F neither increased the number of erythroid bursts nor changed the titration pattern. These data indicate that the relative amount of MuLV does not significantly influence either the number of erythroid bursts or the titration patterns. In other experiments (data not shown) up to  $10^5$  PFU/ml of MuLV-F were added and still no effect on the number of erythroid bursts was observed.

**Time Course of Helper-Deficient FVP-Induced Burst Formation.** In the titration experiments described above, erythroid bursts were scored at 5 days post infection. We considered the possibility that MuLV might have an enhancing effect at an earlier or later time. Therefore, we examined the time course of erythroid burst formation by a low (1:2) and high (1:20) dilution of helper-deficient FVP with or without added

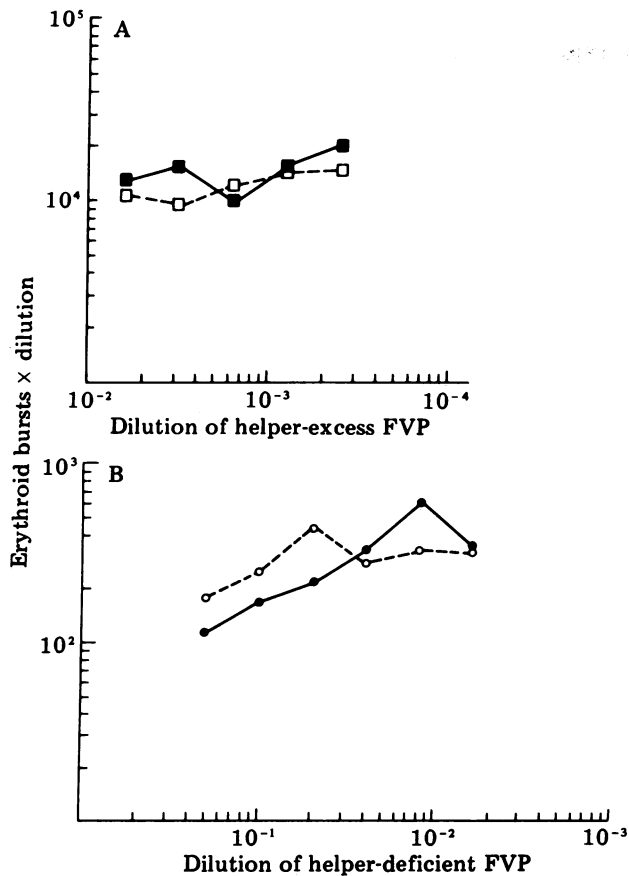


FIG. 2. Kinetics of erythroid burst formation *in vitro* by FVP. Bone marrow cells ( $4 \times 10^6$ ) were infected *in vitro* with FVP in the presence or absence of helper MuLV-F ( $10^4$  PFU/ml). (A) Helper-excess FVP without ( $\square$ ) or with added MuLV-F ( $\blacksquare$ ). (B) Helper-deficient FVP without ( $\circ$ ) or with added MuLV-F ( $\bullet$ ). Each point represents the mean of 3 cultures.

MuLV-F. The cultures were scored for erythroid bursts daily over an 8-day period (Fig. 3). In all cases, the time course of erythroid burst formation was similar to that published earlier (15), and the maximum numbers were observed at days 4–6 after infection. Thus, addition of MuLV-F neither altered the time course nor enhanced burst formation.

**Kinetics of SFFV Production by Hematopoietic Cells *in Vitro*.** Because MuLV-F had no effect on burst formation *in vitro*, a positive control experiment was performed to ensure that MuLV-F was active in this culture system and that sufficient dilution of the helper-deficient FVP preparation had been made to visualize multiple-hit kinetics if such were present. SFFV is replication-defective, and production of progeny SFFV by hematopoietic cells requires a dual infection with both MuLV and SFFV (8). Therefore, one would predict two-hit kinetics of SFFV production when helper-deficient preparations are used for infection of the hematopoietic cells and production of SFFV is measured. To test this, a helper-deficient preparation was appropriately titrated for erythroid burst transformation *in vitro*. On day 5, the cultured hematopoietic cells were collected and virus production was measured by cocultivation of the hematopoietic cells with SC-1 cells. At the end of this procedure, the tissue culture supernatants were harvested and assayed for spleen focus-forming ability in BALB/c mice. The titration patterns shown in Fig. 4 indicate that SFFV production *in vitro*, by hematopoietic cells infected with varying dilutions of helper-deficient FVP, displayed a multihit titration pattern. When MuLV-F was added to the

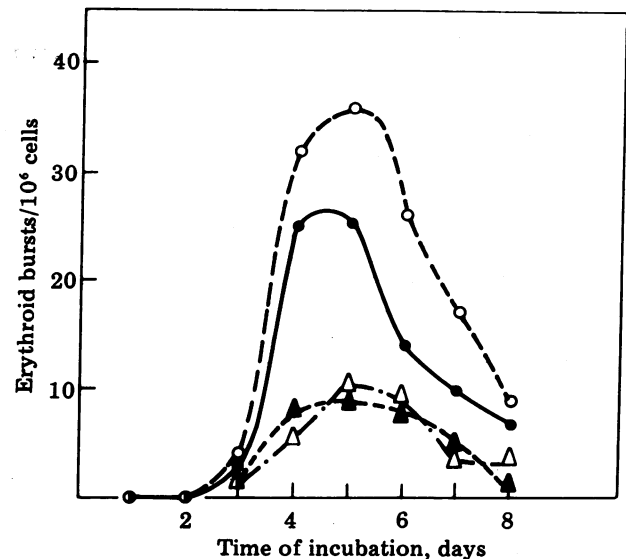


FIG. 3. Time course of erythroid burst formation by helper-deficient FVP in the presence or absence of exogenous helper MuLV. Bone marrow cells ( $4 \times 10^6$ ) were treated with two dilutions of the helper-deficient FVP stocks in the presence or absence of exogenous helper MuLV-F ( $10^4$  PFU/ml). FVP 1:20 diluted without ( $\circ$ ) or with ( $\bullet$ ) MuLV-F; FVP 1:20 diluted without ( $\Delta$ ) or with ( $\blacktriangle$ ) MuLV-F. Triplicate cultures were scored daily for erythroid bursts.

helper-deficient FVP preparations during the initial infection of the hematopoietic cells *in vitro*, the SFFV production curve was converted to one-hit kinetics, and highly significant enhancement for SFFV production was observed. These SFFV production experiments were done four times with essentially the same results. We conclude from these data that SFFV replication is strongly influenced by the amount of MuLV

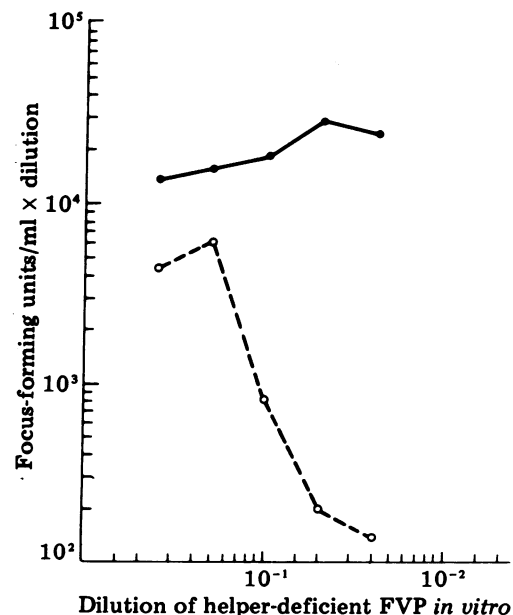


FIG. 4. Kinetics of SFFV production by hematopoietic cells *in vitro*. Bone marrow cells ( $4 \times 10^6$ ) were infected with the indicated dilutions of FVP stock in the presence ( $\bullet$ ) or absence ( $\circ$ ) of helper-MuLV-F ( $10^4$  PFU/ml). After 6 days of culture in methylcellulose, the cells were collected, washed, and cocultivated with SC-1 cells as described in *Methods*. After one passage, the culture fluids from the infected SC-1 cells were harvested and assayed in the spleen focus-forming assay. To insure optimum detection of the SFFV produced in culture, helper MuLV-F was added to SC-1 culture fluids immediately before injection into mice.

present at the time of virus infection and that the helper-deficient virus preparation was diluted sufficiently to observe multihit kinetics.

## DISCUSSION

We have reported that erythroid transformation of hematopoietic cells by the Friend virus complex (SFFV and MuLV-F) *in vitro* leads to the appearance of erythroid bursts without the addition of erythropoietin (14–16). The kinetic experiments reported here provide evidence that the replication-defective SFFV is competent for this *in vitro* erythroid transformation and does not require the presence of MuLV.

Historically, several endpoints have been used as an index of transformation by FVP. These include increased spleen weight (20), increased  $^{59}\text{Fe}$  incorporation into the hemoglobin of spleen erythroblasts (21, 22), spleen focus formation (3), and the generation of erythroleukemic cells (23, 24), which proliferate in culture indefinitely and differentiate into erythroblasts when a variety of chemicals (such as dimethyl sulfoxide) are added to the growth medium. Another manifestation of FVP infection and transformation has been reported by several laboratories (4–7). These studies have shown that FVP infection of mice leads to the formation of infected spleen and bone marrow cells that undergo erythroid differentiation in culture and synthesize hemoglobin in the absence of added erythropoietin. Using  $^{59}\text{Fe}$  incorporation as an index of hemoglobin synthesis, we established that the total time course for this expression of hemoglobin synthesis *in vitro*, by hematopoietic cells from mice infected with FVP *in vivo*, was 4–6 days (6). Since then, conditions have been found that allow bone marrow cells to become infected *in vitro* with FVP and to undergo erythroid differentiation and synthesize hemoglobin without addition of erythropoietin (14, 15). The virus-induced erythroid transformation can be quantitated either by measuring hemoglobin synthesis or by counting the number of clusters (bursts) of erythroid colonies. With either measure, the time course of erythroid differentiation was similar whether the hematopoietic cells were infected and transformed *in vivo* or *in vitro*. Therefore, we believe that the *in vitro* transformation system is representative of the early erythroblastosis *in vivo*.

To approach a molecular understanding of the transformation of hematopoietic cells by FVP, it is important, if not essential, to understand whether SFFV alone or both MuLV and SFFV participate in the production of the transformation. In our examination of numerous uncloned FVP preparations, we observed a constant association between focus-forming ability *in vivo* and burst-forming ability *in vitro* (14–16). Although SFFV does not transform fibroblasts *in vitro*, the SFFV genome was recently isolated in nonproducer fibroblasts of both rat and mouse origin (25). This accomplishment made it possible for us to prepare SFFV pseudotypes by superinfecting the nonproducer cells with a variety of MuLVs. When the various pseudotypes were tested for erythroid burst-forming activity, we again observed a constant association between spleen focus formation and burst formation (16). The MuLVs which were used to rescue SFFV from the nonproducer cells did not induce spleen focus formation *in vivo* or erythroid burst formation *in vitro*. Thus, there is ample evidence that the SFFV is essential for the erythroid transformation *in vitro*, just as there has been ample evidence that SFFV is required for erythroid transformation *in vivo* (26).

The possible role of MuLV in this *in vitro* transformation was the subject of the present study. If pure SFFV were available, a direct test of the need for helper MuLV in the erythroid transformation would be possible. However, SFFV is replication-defective and cannot be propagated without MuLV. Al-

though one report (27) of a physical separation and purification of SFFV, free of MuLV, has appeared, our attempts to remove all MuLV from FVP stocks by these techniques have been unsuccessful. As an alternative means of assessing the role of MuLV in erythroid burst transformation, we compared the *in vivo* and *in vitro* titration kinetics of two FVP preparations—one that is helper-deficient and one that contains an excess of helper MuLV. When the helper-deficient preparation was titrated for spleen focus-forming ability *in vivo*, a multiple-hit kinetic pattern was observed. Addition of exogenous helper MuLV to this preparation, prior to injection into mice, resulted in an increase in the number of spleen foci and converted the multiple-hit pattern to a single-hit pattern. This result was expected because spleen focus formation has been shown to be dependent on virus replication and secondary infection of neighboring cells.

In contrast to the *in vivo* results, the same preparation of helper-deficient FVP yielded single-hit kinetics when titrated in the *in vitro* erythroid transformation system. Addition of exogenous MuLV to the helper-deficient FVP prior to its assay *in vitro* did not increase the number of erythroid bursts formed. The single-hit kinetics and the absence of an effect of added MuLV indicate that the MuLV is not necessary for the erythroid transformation that produces erythroid bursts *in vitro*. We considered the possibility that the added MuLV might not be effective in this *in vitro* system or that the helper-deficient FVP might not have been diluted sufficiently to reveal multiple-hit kinetics; consequently, we examined SFFV replication in these cultures. We found that although SFFV appeared to be competent for the erythroid transformation, its defectiveness for replication and its dependence on MuLV for this function could readily be demonstrated in replicate marrow cell cultures. The production of SFFV by hematopoietic cells followed multihit kinetics, and the addition of exogenous helper virus produced an enhancement of SFFV production with a conversion of the multiple-hit pattern to a single-hit pattern. Whereas helper MuLV clearly is needed for SFFV replication, these data add further support to the conclusion that MuLV is not necessary for the transformation leading to erythroid burst formation. This work does not rule out the possibility that MuLV-derived genetic information is involved in the transformation by SFFV. Our work only indicates that exogenous helper virus is not required for the direct transformation of erythroid cells *in vitro* by a defective particle that is an already manufactured recombinant that includes MuLV and SFFV sequences.

MSV, another replication-defective transforming virus, has been shown to transform fibroblasts without the aid of helper MuLV. Uninfected fibroblasts exhibit contact inhibition in culture and cease dividing when a confluent monolayer of cells is achieved. Fibroblasts exposed to MSV with MuLV were not contact-inhibited and grew over one another, producing multiple foci of transformed cells that were microscopically visible at 4–5 days after infection. Hartley and Rowe (19) demonstrated that this focus formation followed multiple-hit kinetics and was significantly inhibited by addition to the cultures of virus antiserum to inhibit virus spread. Aaronson *et al.* (28) subsequently confirmed this finding and observed that under the appropriate growth conditions, they could identify small foci of transformed cells, which appeared at a later time. The formation of these small and delayed foci followed a one-hit titration pattern and resulted from the proliferation of fibroblasts transformed by MSV without the MuLV. The most convincing evidence that MSV is competent for transformation came with the isolation of clones of transformed cells that contained the MSV genome but did not produce virus (29).

The conclusion that SFFV can cause erythroid transforma-

tion by itself predicts that it should be possible to isolate SFFV-transformed, nonproducer erythroid colonies that carry the SFFV genome but do not produce virus. In attempts to identify nonproducer erythroid cells from these hematopoietic cultures, one difficulty encountered is that at least some hematopoietic cells transformed by SFFV are stimulated to terminally differentiate and, therefore, have a limited lifespan in culture. Repeated attempts to cultivate these cells and form continuous lines so far have not succeeded. However, we have plated single FVP-erythroid bursts onto SC-1 cells and have found that some erythroid bursts produced MuLV and SFFV whereas others did not. Further studies are necessary to determine if the latter have a reasonable SFFV.

We thank Judi Luna for excellent technical assistance. This project is supported in part by National Institutes of Health Grants AM15555 (S.B.K.) and CA26306 (W.D.H.) and the Brownlee O. Currey Research Fund. D.H. is a Leukemia Society Special Fellow.

1. Friend, C. (1957) *J. Exp. Med.* **105**, 307-318.
2. Mirand, E. A. (1968) *Ann. N.Y. Acad. Sci.* **149**, 486-496.
3. Axelrad, A. A. & Steeves, R. A. (1964) *Virology* **24**, 513-518.
4. Horoszewicz, J. S., Leong, S. S. & Carter, W. A. (1975) *J. Natl. Cancer Inst.* **54**, 265-267.
5. Hankins, W. D. & Krantz, S. B. (1975) *Nature (London)* **253**, 731-732.
6. Hankins, W. D., Rosenblatt P. & Krantz, S. B. (1977) *J. Natl. Cancer Inst.* **59** (1), 107-111.
7. Liao, S. K. & Axelrad, A. A. (1975) *Int. J. Cancer* **15**, 467-482.
8. Steeves, R. A., Eckner, R. J., Bennett, M., Mirand, E. A. & Trudel, P. J. (1971) *J. Natl. Cancer Inst.* **46**, 1209-1217.
9. Dawson, P. J., Tacke, R. B. & Fieldsteel, A. H. (1968) *Br. J. Cancer* **22**, 569-576.
10. Troxler, D. H., Boyars, J. K., Parks, W. P. & Scolnick, E. M. (1977) *J. Virol.* **22**, 361-372.
11. Steeves, R. A. & Eckner, R. J. (1970) *J. Natl. Cancer Inst.* **44**, 587-594.
12. Steinheider, G. & Steeves, R. (1978) *Leukemia Res.* **2**, 197-200.
13. Wendling, R. & Tambourin, P. E. (1978) *Int. J. Cancer* **22**, 479-486.
14. Hankins, W. D., Kost, T. A., Koury, M. J. & Krantz, S. B. (1978) *Nature (London)* **276**, 506-508.
15. Hankins, W. D., Krantz, S. B., Kost, T. A. & Koury, M. J. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **44**, in press.
16. Hankins, W. D. & Troxler, D. (1980) in *In Vivo and In Vitro Erythropoiesis: The Friend System*, ed. Rossi, G. (Elsevier/North-Holland, Amsterdam), 151-161.
17. Rowe, W. P., Pugh, W. E. & Hartley, J. W. (1979) *Virology* **42**, 1136-1139.
18. Bernstein, A., Mak, T. W. & Stephenson, J. R. (1977) *Cell* **12**, 287-294.
19. Hartley, J. W. & Rowe, W. P. (1966) *Proc. Natl. Acad. Sci.* **55**, 780-786.
20. Rowe, W. P. & Brodsky, I. (1959) *J. Natl. Cancer Inst.* **23**, 1239-1248.
21. Tambourin, P. & Wendling, F. (1971) *Nature (London) New Biol.* **234**, 230-233.
22. Hankins, W. D. & Krantz, S. B. (1974) *J. Natl. Cancer Inst.* **52**, 1223-1229.
23. Friend, C., Scher, W., Holland, J. G. & Solo, T. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 378-382.
24. Ostertag, W., Melderis, H., Steinheider, G., Kluge, N. & Dube, S. K. (1972) *Nature (London) New Biol.* **239**, 231-234.
25. Troxler, D., Parks, W. P., Vass, W. C. & Scolnick, E. M. (1977) *Virology* **76**, 602-615.
26. Steeves, R. A. (1975) *J. Natl. Cancer Inst.* **54**, 297-298.
27. Eckner, R. J. & Hettrick, K. L. (1977) *J. Virol.* **24**, 383-396.
28. Aaronson, S. A., Jainchill, J. L. & Todaro, G. J. (1970) *Proc. Natl. Acad. Sci. USA* **66**, 1235-1243.
29. Bassin, R. H., Tuttle, N. & Fischinger, P. J. (1970) *Int. J. Cancer* **6**, 95-104.