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Presence of Target Cells for Friend VIRUS (FV^P) in the Liver and Spleen of Early Postnatal Mice (39657)[1]

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Within 72 hr after infection of susceptible mice with a polycythemia-inducing strain of Friend virus (FV^P), transformation of certain hemopoietic cells can be detected by their newly acquired capacity to proliferate and form tumor colonies in the spleens of genetically compatible recipients (1-3). Recent studies have indicated that the target cell for this transforming effect of FV^P is a cell in the erythroid series (4-6), although other experimentation had led other investigators to suggest that the cell may be identified with the hemopoietic stem cell (colony-forming unit, CFU_s) (7, 8). Our own studies used experimentally manipulated hemopoietic cell populations, i.e., treatment with Myleran, to suppress the hemopoietic colony-forming unit (CFU_s) population. By these methods it is possible to dissect the target cell of the virus, shown by its tumor colony-forming capacity, from the CFU_s population. It is desirable, however, to demonstrate, *in vivo,* the separation of the target cell from the CFU_s population without pharmacological intervention. This opportunity is available naturally, presented in neonatal mice where the hemopoietic activities of the spleen and liver are increasing and decreasing, respectively, at this time (9, 10). These two tissues have been used as target organs for the transforming effect of FV^P in order to establish if the development of a CFU_s population corresponds with the increased availability of those target cells for Friend virus, demonstrated by their ability to form tumor colonies; and correlatively, if loss of a CFU_s population leads to a decrease in availability of such target cells.

Materials and methods. Mice. Mice used in these studies were obtained from the animal production unit of Roswell Park Memorial Institute. DBA/2Cr and B6D2F1 mice were used throughout the studies. Recipient mice for irradiation studies were 3- to 5-months old, as were the B6D2F1 mice used as recipients in the tumor colony-forming unit (tCFU) assay. Postnatal mice were used at the ages indicated in the appropriate studies.

CFU assay. Cell suspensions of spleen and liver were made in phosphate-buffered saline (PBS), diluted to appropriate concentrations, and injected via a lateral tail vein into supralethally-irradiated recipient mice. X-Irradiation was done using a 250-kV Maxitron at 30 mA with 1.0-mm A1 and 0.2-mm Cu filtrations and a 50-cm source-to-target distance. The normal irradiation rate was 180 rad/min as determined by a Victoreen dosimeter. A total of 900 R was administered to animals, and cell suspensions were injected into recipient mice within 2 hr after irradiation. Animals were sacrificed 9 days following irradiation and cell transplantation, and spleens were removed and fixed in Bouin's fluid. Macroscopic colonies were counted.

Virus. The Friend virus used in these studies has been previously described (11), and was passaged in DBA/2 (N-type) mice. Cell-free filtrates of spleens from leukemic mice were maintained under liquid nitrogen. Virus doses were expressed in focus-forming units, FFU (12). To achieve the high concentrations of virus used in these studies, 20% filtrates were used directly, and intraperitoneal injections were made. Stocks were titered routinely to insure uniform doses of focus-forming units/assay. At the concentration used, virus titer was not a limiting factor (See Results).

tCFU assay. Susceptibility to the disease induced by FV^P is controlled by several genes. B6D2F1 mice [F_1 progeny of C57Bl/

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6Ja (B-type) and DBA/2 (N-type) parents] are heterozygous at the Fv-1 locus (Fv-$1^{n/b}$) and are resistant to infection with the N-tropic helper virus of FV^P. Cells, transformed by FV^P from DBA/2 mice (Fv-$1^{n/n}$), will grow in spleens of B6D2F1 mice. The expression of the defective, helper-dependent SFFV of FV^P is substantially suppressed in the B6D2F1 assay animal, and the enumeration of tumor colonies is, thereby, not obscured or confused by the simultaneous induction of the macroscopically indistinguishable foci (13, 14). We routinely used a 3-day interval between infection with virus and harvest. Spleen and liver cell suspensions were prepared as before. Cell suspensions were administered via a lateral tail vein. Assay mice were sacrificed at 9 days; the spleens were removed and placed into Bouin's fluid, and macroscopically visible tumor colonies were enumerated. Data are presented for the day of virus inoculation, although cells were not obtained for tCFU assay until 3 days later. Supernatant fluids of cell suspensions used for tCFU assay were routinely assayed in DBA/2 mice for residual FFU and, except in suspensions with high numbers of tCFU, the focus-forming activity of supernatant fluids was negligible.

Results. Hemopoietic colony-forming units (CFU_s) are present in the spleens of newborn mice. Detected numbers ranged from 400–600/spleen (Fig. 1). This represented a frequency of approximately 300/10^6 spleen cells. The total number of CFU_s increases with age as the spleen expands in size to a content in excess of 2000 total CFU/spleen and a frequency approaching 1/10^5 spleen cells (Fig. 2). During this period of time, detectable target cells for Friend virus, demonstrable as tCFU, begin at very low, almost undetectable levels (Figs. 1 and 2). However, over the ensuing week to 10 days, the frequency and number of target cells for the virus increase rapidly. It is apparent, especially, that the pattern of occurrence of the tCFU target cell is substantially different from the occurrence of CFU_s, both with regard to total CFU and tCFU/spleen, as well as CFU/10^6 and tCFU/10^6 spleen cells.

This distinction is consistent with the

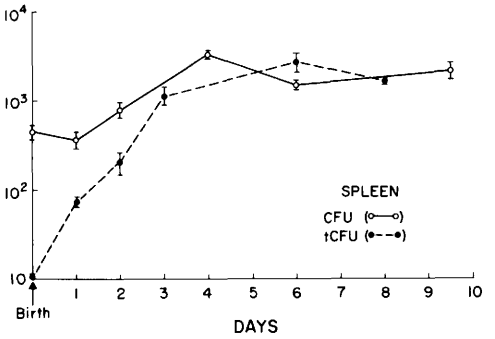

FIG. 1. Hemopoietic spleen colony-forming units (CFU_s) and FV^P-transformed tumor colony-forming units (tCFU) in spleens of postnatal DBA/2 mice. CFU_s determined in supralethally-irradiated DBA/2 and B6D2F1 recipients. Distinction not made between the two host strains; coefficient of correlation >0.96. The tCFU are determined in histocompatible virus-resistant B6D2F1 mice. Spleen cells from FV^P-infected DBA/2 mice were assayed for tCFU content 3 days after intraperitoneal inoculation of hosts with 2×10^4 FFU of FV^P.

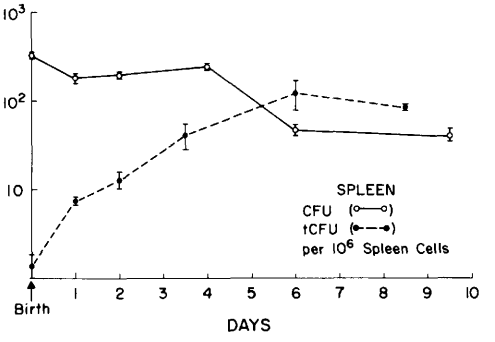

FIG. 2. Frequency of hemopoietic colony-forming units (CFU_s) and FV^P-transformed tumor colony-forming units (tCFU) in spleens of postnatal DBA/2 mice. $CFU_s/10^6$ and tCFU/10^6 spleen cells were presented.

CFU and tCFU content of the liver of neonatal animals (Fig. 3). Beginning with the earliest time studied, CFU_s present in the liver decrease from over 1000 to slightly over 100 CFU/liver. During this period of time, the number of detectable target cells for Friend virus, demonstrable as tCFU increase from slightly more than 10 to in excess of 100 over the same period of time. This again demonstrates a significant and essential difference in the kinetics of the two populations.

To establish that virus concentration was not a limiting factor in the results presented,

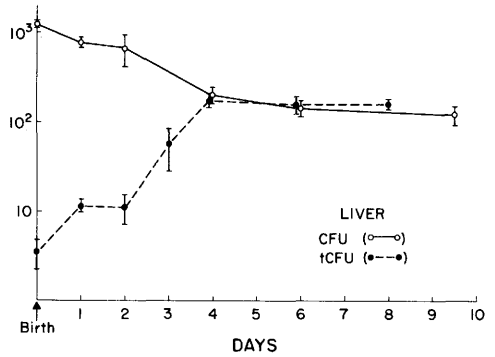

FIG. 3. Hemopoietic spleen colony-forming units (CFU_s) and FV^P-transformed tumor colony-forming units (tCFU) in livers of postnatal DBA/2 mice. CFU_s determined in supralethally-irradiated B6D2F1 and DBA/2 recipients. Distinction not made between the two host strains; coefficient of correlation >0.89. The tCFU are determined in histocompatible virus-resistant B6D2F1 mice. Liver cells from FV^P-infected DBA/2 mice were assayed for tCFU content 3 days after intraperitoneal inoculation of hosts with 2×10^4 FFU of FV^P.

3-day-old DBA/2 mice were infected with 2×10^4, 2×10^3, or 2×10^2 FFU of FV^P intraperitoneally in 0.1 ml of PBS. The number of tCFU in spleens was determined 3 days later in groups of 4 B6D2F1 mice. The results of three independent studies show that detectable tCFU do not differ significantly between the two higher concentrations; $P > 0.2$ for both tCFU/10^6 spleen cells and total tCFU (Table I). This shows that, at the virus concentration used, the principal variable determining the number of detectable tCFU was the availability of appropriate target cells.

Discussion. It is of interest that, as the spleen and liver, especially the spleen, establish an equilibrium of hematopoietic activity somewhere during the second postnatal week, the frequencies of occurrence of target cells for FV^P and that for colony forming units resemble each other. This is something that had been noticed in previous studies (7, 8) and has led investigators, at times, to presume that the CFU_s population did constitute the target cell population for this effect of the Friend virus.

The superficial similarity of values for CFU_s and tCFU at these times is not necessarily a valid reflection of absolute comparability. CFU_s are only a fraction (approximately 20%) of the total CFU in a population of injected cells. This fraction (f value) may be very different from the f value for tCFU, preventing a meaningful interpretation of direct, absolute quantitative determinations of tCFU and CFU_s.

This does not prevent the relative comparison of changing patterns of numbers of available tCFU target cells at different times in hemopoietic populations with changing CFU_s content. This is conceptually different from following the growth kinetics of CFU_s and tCFU within hemopoietic populations of FV^P-infected mice. The essential comparison is not necessarily whether the growth kinetics of populations of CFU_s and tCFU are identical. Although interesting and important, similarity does not indicate identity. The techniques reported here evaluate the nature of the kinetics of the population that serves as a target for this transforming effect of FV^P. By fixing the time after infection when the population is assayed for tCFU content, variability in tCFU can be attributable only to variations in the number of available target cells at the time of virus inoculation.

More recent studies have indicated that the actual target cell for the virus is more closely associated with erythropoietin-responsive cells (4–6). While these are the conclusions of a number of investigators, each of these studies required experimental manipulations *in vivo* which could lead to

TABLE I. THE EFFECT OF FV^P CONCENTRATION ON THE DETECTABLE NUMBER OF TARGET CELLS FOR FV^P, DEMONSTRABLE AS tCFU IN SPLEENS OF 3-DAY-OLD DBA/2 MICE, 3 DAYS AFTER INTRAPERITONEAL INFECTION WITH VIRUS.[a]

Virus titer (FFU)[b]	tCFU in donor spleen	
	Per 10^6 spleen cells	Total per spleen
2×10^4	66.7 ± 10.9[c]	1398 ± 410[c]
2×10^3	49.8 ± 4.5	840 ± 338
2×10^2	0.6 ± 0.2	10 ± 0.3

[a] tCFU assay done in unirradiated B6D2F1 mice. Spleen cells (10^6) were injected into groups of four recipient mice. Data are a result of three independent studies.

[b] FV^P dose is expressed in focus-forming units (FFU)/0.1 ml injected ip.

[c] Value shown not significantly different from that determined for tCFU in spleens of mice infected with 2×10^3 FFU ($P > 0.2$).

some undefined disturbances in normal physiology. The studies reported here have exploited the normal variations in CFU_s in developing hemopoietic populations.

Although the neonatal mouse is immunologically immature, it is not believed that this feature contributed to the observed results. If anything, it might be that this immaturity would lead to increased susceptibility to FV^P and, thus, an increase in the availability of target cells. Therefore, it is improbable that the reduced numbers of tCFU target cells in spleens and livers at early postnatal times are a consequence of immune-mediated reductions in effective FV^P titer.

We conclude from these studies that the target cell for the Friend virus which results in autonomous growth of a cell type demonstrated by its ability to form tumor colonies in appropriate host mice is a cell which is present in hemopoietic populations normally, but is not the hemopoietic stem cell. We have not endeavored, in these studies, to correlate the population kinetics of the tCFU target cell with the erythropoietic activities of the hemopoietic populations studied.

Summary. The growth dynamics of hemopoietically active liver and spleen of neonatal mice have been used to study the association between hemopoietic stem cells (CFU_s) and a target cell for Friend virus, defined by its capacity, following transformation, for autonomous colonial growth in appropriate host mice (tumor colony forming units, tCFU). The kinetics of tCFU target cells and CFU_s are sufficiently dissimilar to allow the conclusion that they do not constitute identical populations.

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