EXPRESSION OF RESISTANCE TO FRIEND VIRUS-STIMULATED ERYTHROPOIESIS IN BONE MARROW CHIMERAS CONTAINING $Fv\text{-}2^{rr}$ AND $Fv\text{-}2^{ss}$ BONE MARROW

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Friend virus induces massive splenomegaly and erythroblastosis in susceptible mice (1, 2). Certain strains of Friend virus, designated FV-P[1] for polycythemia (3), cause the rapid accumulation of erythroid precursors that can differentiate in vivo (4, 5) and in vitro (6, 7) in the absence of erythropoietin. Mice infected with these strains of Friend virus experience a tremendous increase in the rate of erythropoiesis (8) and become polycythemic. The component of Friend virus stocks that is thought to be responsible for inducing polycythemia is a replication-defective virus called spleen focus-forming virus (SFFV; 9–11). Understanding the mechanism of FV-P-stimulated, erythropoietin-independent erythropoiesis should provide fundamental insights into erythroid differentiation and viral leukemogenesis.

Mouse strains that carry the $Fv\text{-}2^r$ allele (12) are strongly resistant to Friend virus-stimulated erythropoiesis (13, 14). The mechanism of this resistance is not well understood. On the one hand, $Fv\text{-}2$ controls the replication of SFFV in vivo (13) and in long-term bone marrow cultures (15), although not in fibroblasts (16). Restricted replication of SFFV in $Fv\text{-}2^r$ mice could explain their relative resistance to virus-stimulated erythropoiesis. On the other hand, the $Fv\text{-}2$ gene also plays a role in normal erythropoiesis, controlling the proportion of early erythroid precursors synthesizing DNA (17), and regulating the expression of virus-related RNA (18) and a virus-related differentiation antigen in uninfected hemopoietic cells (19). These pleiotropic effects of $Fv\text{-}2$ on normal erythropoiesis could also be related to the mechanism of resistance to virus-stimulated erythropoiesis. The experiments reported here were designed to investigate whether $Fv\text{-}2^r$ restriction of SFFV replication, or some other effect of the $Fv\text{-}2^r$ gene, is responsible for resistance of $Fv\text{-}2^r$ mice to Friend virus-stimulated erythropoiesis.

In these experiments, bone marrow chimeras containing mixtures of $Fv\text{-}2^{rr}$ and $Fv\text{-}2^{ss}$ bone marrow were constructed. The bone marrow donors were chosen to differ in hemoglobin type so that the proportion of erythrocytes derived from each donor in the chimera could be determined by hemoglobin electrophoresis. These chimeric mice

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were infected with FV-P, and the effect of the virus on erythropoiesis derived from each donor marrow was determined at various times after infection.

Previous experiments have shown that $Fv\text{-}2$ phenotype is determined by the transplanted bone marrow cells in radiation chimeras. Thus, transplantation of $Fv\text{-}2^{ss}$ bone marrow into lethally irradiated $Fv\text{-}2^{rr}$ mice renders them susceptible to Friend virus, whereas transplantation of $Fv\text{-}2^{rr}$ bone marrow into $Fv\text{-}2^{ss}$ mice renders them resistant (14, 20). Radiation chimeras containing mixtures of $Fv\text{-}2^{rr}$ and $Fv\text{-}2^{ss}$ bone marrow are susceptible to Friend virus as determined by spleen focus formation (14), as are F_1 mice of $Fv\text{-}2^{rr} \times Fv\text{-}2^{ss}$ crosses. Because spleen foci are associated with proliferating cells of the erythroid lineage (9), it seemed likely that chimeras containing mixtures of $Fv\text{-}2^{rr}$ and $Fv\text{-}2^{ss}$ bone marrow would be susceptible to virus-stimulated erythropoiesis.

The experimental results show that chimeras containing mixtures of $Fv\text{-}2^{rr}$ and $Fv\text{-}2^{ss}$ bone marrow are indeed susceptible to virus-stimulated erythropoiesis, but that the $Fv\text{-}2^{rr}$ bone marrow maintains its resistance in the chimeras. Because both bone marrows are exposed to the same amount of SFFV in these mice, the results indicate that $Fv\text{-}2^{rr}$ bone marrow is resistant to virus-stimulated erythropoiesis even when exposed to an amount of SFFV sufficient to stimulate $Fv\text{-}2^{ss}$ erythroid precursors. This implies that $Fv\text{-}2^{rr}$ mice are resistant to virus-stimulated erythropoiesis for reasons other than poor replication of SFFV.

Materials and Methods

Mice. DBA/2, B10.D2, and (C57BL/10 × DBA/2)F_1 (BDF$_1$) mice were obtained from the Imperial Cancer Research Fund Laboratories.

Virus. NB-tropic (21) FV-P was a gift from F. Lilly (Department of Genetics, Albert Einstein College of Medicine) and was routinely passaged as a 10% spleen homogenate in DBA/2 or BDF$_1$ mice. This virus gave approximately equal spleen focus-forming titers (9) in DBA/2 and BDF$_1$ mice.

Radiation Chimeras. 3–5-mo-old male DBA/2 or female B10.D2 mice were exposed to 1,000 ± 50 rad (240 kV, 14.5 mA, 130 rad/min, 18″ from x-ray source to target). Immediately after irradiation, mice were transfused through the tail vein with 7×10^6 bone marrow cells from DBA/2 donors, B10.D2 donors, or mixtures of bone marrow cells from the two donors. Irradiated controls that were not transfused with bone marrow all died within 2 wk. Mice transplanted with viable bone marrow cells had a survival of ~85% at 1 mo with sporadic deaths for 4–6 mo thereafter, at which time the experiments were terminated.

Phenylhydrazine (PHZ) Treatment. Mice were injected with 0.8 mg of PHZ intraperitoneally or subcutaneously at the indicated times after bone marrow transplantation and/or virus infection.

Routine Hematology. Mice were bled from the tail vein. Hematocrits were measured using heparinized microhematocrit tubes. Reticulocytes were stained with 1% brilliant cresyl blue (22).

Hemoglobin Electrophoresis. Erythrocytes were washed three times with normal saline and lysed in 15 vol of solution containing 67 mM cystamine dihydrochloride (Aldrich Chemical Co., Inc., Milwaukee, Wis.), 103 mM dithiothreitol, and 0.25% NH$_3$OH (23). Membranes were removed by centrifugation. Hemolysates were electrophoresed for 2–4 h at 225 V on cellulose acetate strips (Shandon "cellogram" [Shandon Southern Instruments, Inc., Sewickley, Pa.] or Whatman "cellogel" [Whatman, Inc., Clifton, N. J.]) using a buffer containing 180 mM Tris, 100 mM boric acid, and 2 mM EDTA, pH 8.9. Some gels were stained with 1% Ponceau S in 45% methanol-5% acetic acid and destained in 5% acetic acid. Other gels were air dried and autoradiographed using Kodak XR-5 film (Eastman Kodak Co., Rochester, N. Y.).

^{59}Fe Labeling. Intact mice were injected intravenously or intraperitoneally with 1–10 μCi of freshly buffered ^{59}FeCl$_3$ (2–20 μCi/μg; Amersham Corp., Arlington Heights, Ill.) and bled 2 d

later. In some experiments, reticulocytes were labeled in vitro by adding 20 μl of whole blood to a solution containing 0.45 ml dialyzed fetal calf serum, 0.45 ml α medium (Flow Laboratories, Inc., Rockville, Md.), and 0.1 ml (5 μCi) of freshly buffered ^{59}FeCl$_3$. The mixture was incubated at 37°C for 2–8 h. Radiolabeled erythrocytes were washed three to six times with Dulbecco's modified Eagle's medium to remove excess label and then prepared for hemoglobin electrophoresis as above. Hemolysates were kept at 4°C and electrophoresed within 12 h.

Quantitation of the Relative Amount of Each Hemoglobin Type.   Individual hemoglobin bands were cut from the cellulose acetate strips and eluted in 1 ml of distilled H$_2$O. Absorbance of each eluate was measured spectrophotometrically at 415 nm. The relative amount of each hemoglobin type in a mixture was calculated as the ratio of absorbance of the eluate from that band to the total hemoglobin absorbance (24). Radioactivity was measured in a Beckman liquid scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.). The relative amount of newly synthesized hemoglobin of each type was calculated as the ratio of dpm in the eluate from a particular hemoglobin band to the total dpm from all hemoglobin bands. Aliquots of 5–60 μl of hemolysate were used for each sample (applied as a linear streak 5 μl/cm) so that the total number of hemoglobin-associated dpm was about 2,000 per sample.

Statistics.   The proportion of total or newly synthesized hemoglobin of a given type was determined for each mouse. These values were averaged for mice within each group. Statistical significance was calculated using the two-tailed Student's t test.

Results

DBA/2 and B10.D2 mice were chosen to make the chimeras because, in addition to differing at *Fv-2* and hemoglobin (*Hbb*) loci, these strains are identical at *H-2*, the major histocompatibility locus; both strains have the *H-2^d* haplotype. *H-2* identity was important in order to minimize graft-vs.-host or "graft-vs.-graft" disease in the chimeras. B10.D2 mice are resistant to Friend virus at *Fv-2* (*Fv-2^{rr}*) and have the "single" hemoglobin type, *Hbb^{ss}*; DBA/2 mice are susceptible to Friend virus at *Fv-2* (*Fv-2^{ss}*) and have the "diffuse" hemoglobin type, *Hbb^{dd}*.

The hemoglobins of B10.D2 and DBA/2 mice can be clearly distinguished by electrophoresis (23) as shown in Fig. 1. The proportion of each type of hemoglobin in a mixture can be analyzed quantitatively by electrophoresis followed by elution of hemoglobin bands and measurement of absorption at 415 nm, as described in Materials and Methods. For mixtures containing at least 15% of either type of hemoglobin, this method gave reproducible results to within 5% (data not shown).

To measure the relative amounts of newly synthesized hemoglobin of different types in a mixture, reticulocytes were labeled in vivo or in vitro with ^{59}Fe (see Materials and Methods). Hemolysates were then electrophoresed and the dpm of ^{59}Fe in the various hemoglobin bands were compared. This method of measuring relative
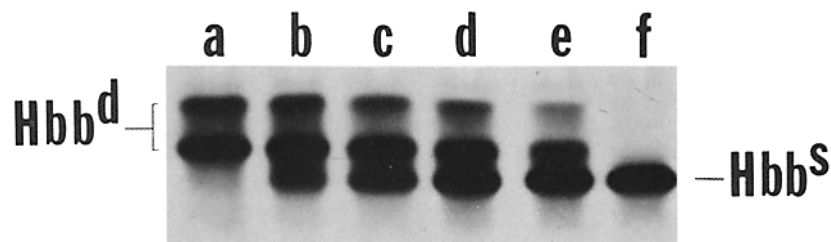

Fig. 1.   Cellulose acetate electrophoresis of hemoglobins from DBA/2 and B10.D2 mice. Hemolysates were prepared from mixtures of whole blood from normal DBA/2 and B10.D2 mice, mixed in the ratios: (a) 100% DBA/2; (b) 80% DBA/2:20% B10.D2; (c) 60% DBA/2:40% B10.D2; (d) 40% DBA/2:60% B10.D2; (e) 20% DBA/2:80% B10.D2; and (f) 100% B10.D2. The electrophoretogram was stained with 1% Ponceau S.

amounts of newly synthesized hemoglobin of different types is very simple, but has a potential disadvantage. If there were a significant iron exchange between hemoglobin molecules in a hemolysate (25), one could not use the proportion of ^{59}Fe in one hemoglobin band to measure its relative synthesis rate. To test whether iron exchange would pose a problem, the following control experiments were done.

In one set of experiments, ^{59}Fe-labeled erythrocytes of each hemoglobin type were mixed with unlabeled erythrocytes of the other hemoglobin type. The mixtures were hemolyzed and electrophoresed after incubation for various times. It was found that hemolysates kept at 4°C and electrophoresed within 12 h showed <10% incorporation of radioactivity into the originally unlabeled hemoglobin type. Longer incubations and higher temperatures resulted in greater transfer of label. Therefore, to minimize iron exchange, hemolysates were kept cold and electrophoresed within 12 h.

A more physiological control experiment is shown in Fig. 2. DBA/2 mice were lethally irradiated (1,000 ± 50 rad) and transfused with 7 × 10^6 bone marrow cells from DBA/2 donors, B10.D2 donors, or a 1:1 mixture of bone marrow cells from the two strains. 1 mo after transplantation, the recipients were injected with 1 μCi of ^{59}Fe intravenously and bled 2 d later. Because mouse erythrocytes have a life span of about 50 d (26), at 1 mo after bone marrow transplantation, approximately half of the circulating erythrocytes should be of recipient type, having been formed before the transplant, whereas the remaining erythrocytes should be of donor type, having been synthesized after the transplant. The upper panel of Fig. 2 shows that mice transplanted with B10.D2 bone marrow had ~50% DBA/2-type hemoglobin and 50% B10.D2-type hemoglobin, whereas mice that received a 1:1 mixture of B10.D2 and DBA/2 bone marrow had ~75% DBA/2-type hemoglobin and 25% B10.D2-type hemoglobin, as expected. The lower panel of Fig. 2 is an autoradiogram of the gel
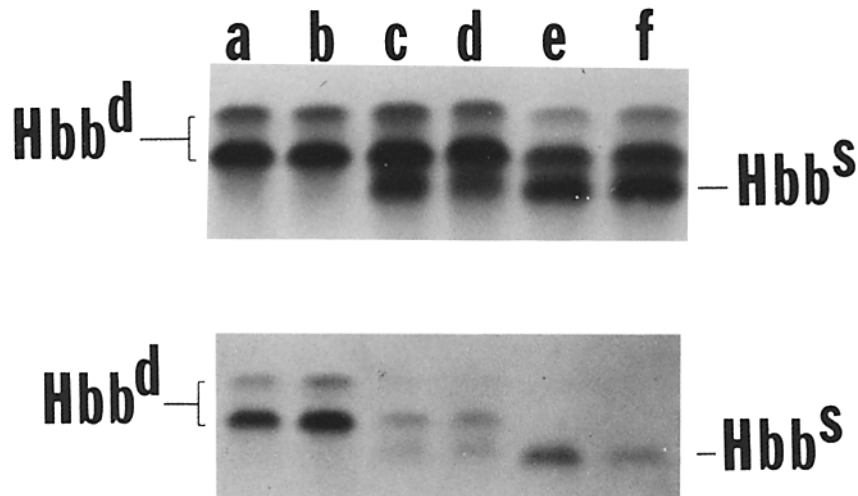

Fig. 2.   Cellulose acetate electrophoresis of hemoglobin from chimeras labeled in vivo 1 mo after bone marrow transplantation. DBA/2 mice were lethally irradiated and transplanted with DBA/2 bone marrow (lanes a and b), B10.D2 bone marrow (lanes e and f), or a 1:1 mixture of DBA/2 plus B10.D2 bone marrow (lanes c and d). 1 mo later, each mouse received 1 μCi of ^{59}Fe intravenously. The upper panel shows a photograph of a Ponceau S-stained gel. The lower panel shows an autoradiograph of the same gel before Ponceau S staining. Each lane contains hemoglobin from a different mouse.

shown in the upper panel. It demonstrates clearly that only the donor-type hemoglobin is radioactive, despite the presence of up to 50% unlabeled recipient-type hemoglobin in the sample. This experiment shows that one can selectively label newly synthesized hemoglobin with ^{59}Fe in chimeras containing both types of hemoglobin. Because iron exchange between labeled and unlabeled hemoglobin molecules did not occur to a significant extent, the method was deemed adequate to measure relative rates of synthesis of different hemoglobin types in chimeras infected with FV-P.

Stability of Hemoglobin Type in Radiation Chimeras. Several groups of DBA/2 and B10.D2 mice were lethally irradiated and transfused with DBA/2, B10.D2, or a 1:1 mixture of DBA/2 and B10.D2 bone marrow. Blood samples were analyzed from 2 to 4 mo after transplantation to determine the proportion of hemoglobin of the two types. Mice that were transplanted with allogeneic bone marrow never showed detectable amounts of syngeneic hemoglobin. Mice that received 1:1 mixtures of DBA/2 and B10.D2 bone marrow contained mixtures of the two hemoglobin types, usually in the range of 30–50% B10.D2 hemoglobin and 70–50% DBA/2 hemoglobin. The reason for the slight predominance of DBA/2 hemoglobin in the chimeras is not known. Because it occurred in both B10.D2 and DBA/2 recipients, it does not represent recovery of endogenous bone marrow. In a few mice followed serially from 2 to 4 mo after transplantation, there was a slight increase in the proportion of DBA/2 hemoglobin with time, on the order of ~5% more DBA/2 hemoglobin per month. This could represent a proliferative advantage of DBA/2 erythroid precursors, or preferential destruction of B10.D2 erythroid precursors or erythrocytes. It should be noted that B10.D2 and DBA/2 mice differ at several minor histocompatibility loci, including erythrocyte antigens (27). A Coombs test was done to investigate the possibility of immune erythrocyte destruction; no Coombs-positive chimeras were found.

Effect of FV-P on Hemoglobin Synthesis in Radiation Chimeras. Groups of chimeras containing mixtures of B10.D2 and DBA/2 bone marrow were infected with 1,000 focus-forming units (FFU) of FV-P and examined for type of hemoglobin being synthesized 1 mo later. As shown in Fig. 3, infected chimeras stopped synthesizing B10.D2 hemoglobin, whereas control chimeras continued to synthesize both types of hemoglobin. This result was independent of whether the irradiated host was DBA/2 or B10.D2.

Quantitative results from this experiment are shown in Table I. In uninfected
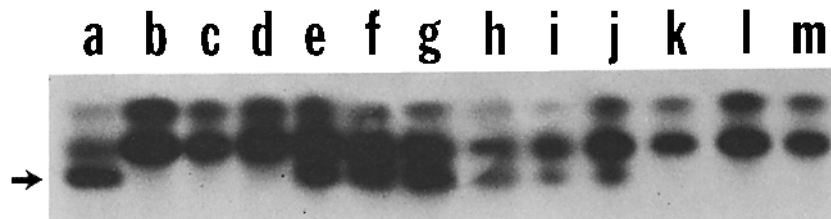

Fig. 3.   Autoradiograph of hemoglobin electrophoresis from mice labeled in vivo with 10 µCi ^{59}Fe. Lane a: BDF$_1$ control. Lanes b–g: B10.D2 mice lethally irradiated and transplanted with a 1:1 mixture of B10.D2 and DBA/2 bone marrow 4 mo earlier. Lanes b–d: chimeras infected with 1,000 FFU of FV-P 1 mo earlier; lanes e–g: uninfected chimeras. Lanes h–m: DBA/2 mice lethally irradiated and transplanted with a 1:1 mixture of DBA/2 and B10.D2 bone marrow 4 mo earlier; lanes h–j: uninfected chimeras; lanes k–m: chimeras infected with 1,000 FFU of FV-P 1 mo earlier. Each lane contains hemoglobin from a different mouse. Arrow indicates *Hbb^s* band.

Table I

Effect of Friend Virus Infection on Synthesis of Hbb[s] in Bone Marrow Chimeras

Treatment group	Number of mice	Hematocrits	Reticulocytes	Total hemoglobin	Newly synthesized hemoglobin
		%	%	% Hbb[s]	% Hbb[s]
I. B10.D2 chimeras	3	48 ± 4	6 ± 1	38 ± 7	36 ± 7
II. B10.D2 chimeras + FV-P	3	54 ± 5*	12 ± 6	28 ± 10	8 ± 1‡
III. DBA/2 chimeras	3	43 ± 2	4 ± 1	28 ± 7	22 ± 6
IV. DBA/2 chimeras + FV-P	3	53 ± 11	10 ± 7	19 ± 1	9 ± 1§

Mice were lethally irradiated and transplanted with a 1:1 mixture of DBA/2 and B10.D2 bone marrow cells 3 mo before the start of the experiment. On day 0, mice in groups II and IV were inoculated with 1,000 FFU of Friend virus intravenously. All mice received 10 μCi of ^{59}Fe intravenously on day 25 and were bled 2 d later. Numbers in the table are mean ± SD of values determined on day 27, unless otherwise indicated.
* Hematocrits on day 17 after infection.
‡ $P < 0.001$ compared with control group.
§ $P < 0.02$ compared with control group.

B10.D2 chimeras, 36% of newly synthesized and 38% of total hemoglobin was of B10.D2 type. Infected chimeras were slightly polycythemic (mean hematocrit = 54%) and reticulocytotic (mean reticulocyte count = 12%) 4 wk after infection. At that time, 28% of the total hemoglobin and <10% of the newly synthesized hemoglobin was of B10.D2 type. The fall in the proportion of B10.D2 hemoglobin in infected animals is consistent with decreased synthesis of B10.D2 hemoglobin in the weeks after infection. Similar results are shown for the DBA/2 chimeras.

As noted above, some uninfected chimeras followed from 2 to 4 mo after transplantation also showed a decrease in the proportion of B10.D2 hemoglobin. This raised the possibility that Friend virus might simply accelerate a normal shift to DBA/2 hemoglobin in the chimeras, perhaps by increasing the rate of erythropoiesis or shortening erythrocyte survival nonspecifically (28). To test whether other agents that shorten erythrocyte survival and stimulate erythropoiesis would accelerate a shift toward DBA/2 hemoglobin, groups of chimeras were given repeated injections of PHZ. PHZ causes acute hemolysis with a compensatory increase in the rate of erythropoiesis. The dose and schedule of PHZ administration were chosen to produce at least as much stimulation of erythropoiesis, as estimated by reticulocyte count, as seen in Friend virus-infected chimeras. Tables II and III show that PHZ does not significantly alter the proportional rate of B10.D2 hemoglobin synthesis in the chimeras (compare groups I and II), whereas Friend virus dramatically reduces it (groups III and IV). We conclude that the effect of Friend virus infection is not the result of a general increase in the rate of erythrocytes synthesis or destruction.

The results so far are consistent with B10.D2 bone marrow remaining resistant to Friend virus-stimulated, erythropoietin-independent erythropoiesis in the chimeras. In such infected polycythemic chimeras, erythropoietin would be expected to be suppressed (4, 5), and in the absence of erythropoietin, B10.D2 erythroid precursors might not be induced to differentiate. To test whether B10.D2 erythroid precursors remained subject to normal erythropoietic stimulation in the infected chimeras, groups of infected animals were given PHZ after they had become polycythemic. As

TABLE II

Effect of PHZ on Synthesis of Hbb^s in Uninfected and Infected B10.D2 Bone Marrow Chimeras

Treatment group	Number of mice	Hematocrits	Reticulocytes	Total hemoglobin	Newly synthesized hemoglobin
		%	%	% Hbb^s	% Hbb^s
I. Control	7	48 ± 2	2 ± 2	39 ± 10	30 ± 5
II. PHZ	5	49 ± 2	41 ± 13	33 ± 7	32 ± 7
III. FV-P (100 FFU)	3	49 ± 5	9 ± 6	32 ± 9	19 ± 6*
IV. FV-P (1,000 FFU)	3	45 ± 12	11 ± 3	40 ± 12	8 ± 1‡
		Before PHZ After PHZ	Before PHZ After PHZ	After PHZ	After PHZ
V. FV-P (100 FFU) + PHZ	2	60 ± 5 39 ± 13	7 ± 2 40 ± 6	33 ± 3	34 ± 6§
FV-P (1,000 FFU) + PHZ	1				

B10.D2 mice were lethally irradiated and transplanted with a 1:1 mixture of DBA/2 and B10.D2 bone marrow cells 2 mo before the start of the experiment. Hemoglobin was labeled in vivo by injecting 10 μCi ^{59}Fe intravenously 2 d before the final bleeding. Group I, control chimeras; group II, chimeras injected with 0.8 mg PHZ intraperitoneally on days 0, 4, 9, 12, 20, and 21, and bled on day 25; group III, chimeras infected with 100 FFU of FV-P on day 0 and bled on day 25; group IV, same as group III, but infected with 1,000 FFU of FV-P; group V, two chimeras infected with 100 FFU and one chimera infected with 1,000 FFU of FV-P on day 0. These mice were bled on day 22 (before PHZ) and then given 0.8 mg PHZ subcutaneously on days 22 and 23 and bled again on day 28 (after PHZ). Numbers in the table represent the mean ± SD.
* $P < 0.05$ compared with controls.
‡ $P < 0.001$ compared with controls.
§ $P < 0.05$ compared with group III.

TABLE III

Effect of PHZ on Synthesis of Hbb^s in Uninfected and Infected DBA/2 Bone Marrow Chimeras

Treatment group	Number of mice	Day tested	Hematocrits	Reticulocytes	Total hemoglobin	Newly synthesized hemoglobin
			%	%	% Hbb^s	% Hbb^s
I. Control	8	14	44 ± 7	4 ± 2	42 ± 9	36 ± 8
II. PHZ	5	14	43 ± 2	26 ± 5	27 ± 8	31 ± 7
III. FV-P	8	14	59 ± 4	24 ± 7	29 ± 4	11 ± 1*
IV. FV-P	4	23	61 ± 4	14 ± 7	21 ± 6	9 ± 2*
V. FV-P + PHZ	6	23	35 ± 3	29 ± 7	22 ± 4	21 ± 4‡

DBA/2 mice were lethally irradiated and transplanted with a 1:1 mixture of DBA/2 and B10.D2 bone marrow cells 2.5 mo before the start of the experiment. At the indicated times after infection (day 0) or after the start of PHZ administration (day 0), chimeras were bled (100 μl from the tail vein) and reticulocytes were labeled in vitro with ^{59}Fe. Group I, control chimeras; group II, chimeras given PHZ (0.8 mg) intraperitoneally on days 0, 1, 6, and 11; group III, eight chimeras infected with 1,000 FFU of FV-P on day 0; group IV, two mice from group III and two additional chimeras infected with 1,000 FFU of FV-P on day 0; group V, six mice from group III given PHZ (0.8 mg) subcutaneously on days 18, 19, and 20. Numbers in the table are mean ± SD.
* $P < 0.001$ compared with control group.
‡ $P < 0.001$ compared with group IV.

shown in Tables II and III (group V), PHZ treatment of infected plethoric chimeras reduced the hematocrit and restored synthesis of B10.D2 hemoglobin.

In most uninfected chimeras, the proportion of newly synthesized hemoglobin of B10.D2 type is slightly less than the proportion of total hemoglobin of B10.D2 type

(Tables I–III). The difference between these proportions was calculated for each chimera. When the data from all chimeras were combined, the average difference was 6.3 ± 1.4% (mean ± SEM). This number is significantly different from zero ($P <$ 0.001). The fact that the proportion of total hemoglobin of B10.D2 type is slightly, but significantly, greater than the proportion of newly synthesized hemoglobin of B10.D2 type, suggests a gradual decrease in the relative rate of synthesis of B10.D2 hemoglobin. Such a decrease is consistent with the observation of a gradual fall in the proportion of B10.D2 hemoglobin in uninfected chimeras followed for 2–4 mo after transplantation (see above).

In animals treated with PHZ, there is closer agreement between the proportion of newly synthesized hemoglobin of B10.D2 type and the proportion of total hemoglobin of B10.D2 type (Tables II and III). The difference between these proportions was calculated for each chimera treated with PHZ. The average difference was found to be 1.0 ± 1.3% (mean ± SEM), not significantly different from zero ($P >$ 0.4). This result is not surprising because PHZ causes massive hemolysis, destroying old erythrocytes; after PHZ treatment, nearly all of the hemoglobin present is newly synthesized. Hence, the proportion of total hemoglobin of B10.D2 type should be the same as the proportion of newly synthesized hemoglobin of B10.D2 type.

It is possible that PHZ treatment could artifactually produce close agreement between the proportion of total and newly synthesized hemoglobin of B10.D2 type. This would be the case if PHZ increased the rate of iron exchange between hemoglobin molecules (for example, by increasing the amount of methemoglobin, a species that facilitates iron exchange) (25, 29). However, this possibility is extremely unlikely because (a) PHZ treatment was always stopped several days before ^{59}Fe labeling and preparation of hemolysates, (b) no increase in methemoglobin was detected (<1%) in blood samples from mice treated 2 d earlier with PHZ, and (c) no increase in iron exchange between labeled and unlabeled hemoglobins was observed when these hemoglobins were obtained from mice treated with PHZ (data not shown).

Discussion

The experiments reported here show that B10.D2 bone marrow remains resistant to Friend virus-stimulated erythropoiesis even when exposed to an amount of SFFV that, in the same chimeric animal, stimulates DBA/2 erythroid precursors. One may conclude that resistance to virus-stimulated erythropoiesis is an intrinsic property of B10.D2 erythroid precursors. This, in turn, implies that B10.D2 mice are resistant to Friend virus-stimulated erythropoiesis for reasons other than poor replication of SFFV.

The experimental results strongly suggest that DBA/2 erythroid precursors become independent of erythropoietin in the infected chimeras, because DBA/2 hemoglobin synthesis continues after the mice have become polycythemic. Previous experiments have shown that erythropoietin, the physiological regulator of erythropoiesis, is undetectable in polycythemic Friend virus-infected mice (4, 5). Our experiments suggest that B10.D2 erythroid precursors remain under erythropoietin control because B10.D2 hemoglobin synthesis stops when infected chimeras become polycythemic, and starts again when the mice are made anemic with PHZ. However, the erythropoietin dependence of B10.D2 erythroid precursors must remain conjectural because

erythropoietin levels were not measured, and PHZ may stimulate erythropoesis by mechanisms other than simply the release of erythropoietin.

The relationship between SFFV replication and virus-stimulated erythropoiesis may be quite complex on a cellular level. For example, it is possible that SFFV must infect erythroid precursors to induce them to differentiate in the absence of erythropoietin. According to this hypothesis, if the $Fv-2^r$ gene prevented SFFV from replicating in hemopoietic cells (15), it could block virus-stimulated erythropoiesis. On the other hand, it has also been suggested that the majority of SFFV produced in vivo may come from rapidly proliferating hemopoietic cells (16). By this hypothesis, if the primary effect of the $Fv-2^r$ gene were to block virus-stimulated erythropoiesis, then poor SFFV replication in the animal would follow secondarily. To investigate these hypotheses, it will be important to determine whether B10.D2 hemopoietic cells in the chimeras are themselves infected with SFFV.

The experimental results provide additional evidence that virus-stimulated erythropoiesis is not mediated by erythropoietin, other humoral factors or cell-cell interactions that substitute for erythropoietin. If such mechanisms were involved in virus-stimulated erythropoiesis, they would be expected to act "in trans" on B10.D2 erythroid precursors in the chimeras, leading to apparent loss of resistance of B10.D2 bone marrow. This was not observed. Of course, the experiments do not rule out the possibility of erythropoietic factors or cell-cell interactions that do not operate across strain barriers, or whose action might be modified by SFFV infection of erythroid precursors.

The experimental results raise may questions about the nature and level of the block to B10.D2 hemoglobin synthesis in the infected chimeras. Is terminal differentiation of B10.D2 erythroid precursors blocked merely by the absence of erythropoietin, or do suppressive factors or interactions exist, as have been suggested for inhibition of normal stem cells in patients with polycythemia vera (30)? Are B10.D2 erythroid precursors increased in number of the bone marrow and/or spleens of infected chimeras? The latter might be suspected because DBA/2 erythroid precursors should be increased in number in the infected chimeras (31), and yet a large fraction of newly synthesized hemoglobin is of B10.D2 type after treatment with PHZ. The application of clonal assays of erythroid precursors to this system should help answer these questions.

Because these experiments were done with strains of mice that differ at many loci, one cannot with assurance attribute the observed effects to particular genes such as $Fv-2$. Thus, although it might be tempting to relate the slow increase in DBA/2 hemoglobin in uninfected chimeras to $Fv-2$-controlled differences in rate of cycling of erythroid precursors (17), one cannot rule out effects due, for example, to minor histocompatibility differences. Similarly, the resistance of B10.D2 bone marrow to Friend virus-stimulated erythropoiesis in the chimeras could be due to other Friend virus resistance genes in the B10 strain background (32, 33). Experiments using congenic strains differing only at $Fv-2$ and Hbb should clarify these points.

Although the chimeras used in these experiments were susceptible to Friend virus-stimulated erythropoiesis, they were clearly less susceptible than nonirradiated DBA/2 controls. This was apparent in the low levels of polycythemia achieved after a substantial dose of SFFV, a reduced degree of splenomegaly in the infected chimeras, and a delay in death from leukemia among chimeras compared with DBA/2 controls

(unpublished observations). The relative resistance of the chimeras to Friend virus could have been contributed by the B10.D2 bone marrow, because the B10 background carries genes that affect the immune response to Friend virus (32, 33).

The experimental system described here is analogous to a rare "experiment of nature": glucose 6-phosphate dehydrogenase (G6PD) heterozygous women with polycythemia vera. Elegant studies of two such women, who are "natural" chimeras for the X chromosome-coded enzyme G6PD, show that erythropoietin-independent erythroid precursors are all of one G6PD type, whereas some erythropoietin-dependent erythroid precursors carry the other G6PD type (30). These studies support the hypothesis that polycythemia vera is a clonal disease and provide a way to study the interaction between the "polycythemia" clone and "normal" stem cells in a single host. In the artificial murine chimeras described here, erythropoietin-independent erythroid precursors carry one hemoglobin type, whereas seemingly erythropoietin-dependent erythroid precursors carry the other hemoglobin type. It should therefore be possible to do similar studies to investigate the interaction between "normal" and "diseased" erythroid precursors in these Friend virus-infected bone marrow chimeras. The animal system has obvious advantages in terms of reproducibility and manipulability compared with the rare human condition for which it serves as a model.

Summary

Bone marrow chimeras were formed containing mixtures of DBA/2 ($Fv-2^{ss}$, Hbb^{dd}) and B10.D2 ($Fv-2^{rr}$, Hbb^{ss}) bone marrow. When these mice were infected with the polycythemia-inducing strain of Friend virus, erythropoiesis was stimulated, but the proportion of B10.D2 hemoglobin fell rapidly and newly synthesized hemoglobin was essentially all of the DBA/2 type. The treatment of infected polycythemic chimeras with phenylhydrazine lowered the hematocrit and restored the synthesis of B10.D2 hemoglobin. These results imply that B10.D2 erythroid precursors are intrinsically resistant to Friend virus-stimulated erythropoiesis. The experiments also suggest that virus-stimulated erythropoiesis is not mediated by a factor or cell-cell interactions, unless such factors or interactions do not act across strain barriers.

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