NOVEL DIFFERENTIATION ANTIGENS ON LATE ERYTHROCYTIC PROGENITOR CELLS DETECTED BY ALLOANTISERA MADE BETWEEN MOUSE STRAINS CONGENIC AT THE Fv-2 LOcus*

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Properties of the late erythrocytic progenitor cells (CFU-E)¹ (1, 2) and of the earlier erythropoietic progenitor cells (BFU-E) (3) have largely been deduced from the behavior of their differentiated progeny in erythrocytic colonies or bursts, rather than from direct studies on the progenitor cells themselves. Because changes in the expression of cell surface molecules are known to occur along the differentiation pathways of several cell types (4, 5), we used an immunological approach to try to identify cell surface molecules characteristic of particular stages of differentiation in the erythrocytic lineage of mice. Such markers, if found, should make it possible to do direct studies on these progenitor cells.

A scheme of reciprocal immunization was chosen to exploit the limited genetic difference between the congenic mouse strains C57BL/6Ut (B6) and B6.S/Ut (B6.S). The B6.S mouse strain had been made congenic with the B6 inbred strain (6) for the purpose of studying the genetic control of the host response to the erythroleukemia-inducing Friend virus (7). The genetic difference between B6 and B6.S was later mapped (8) to the same segment of chromosome 9 as the Fv-2 gene locus, the major determinant of susceptibility or resistance to this virus (9). The B6.S mouse strain carrying the Fv-2' allele is susceptible, whereas the B6 mouse strain, with the Fv-2" allele, is resistant to Friend virus.

Recently (10) it was discovered that the Fv-2 locus functions in uninfected animals as well, where it controls the proportion of erythropoietic progenitor cells BFU-E normally synthesizing DNA. The Fv-2' allele in B6.S is associated with a high proportion of BFU-E in DNA synthesis, and the Fv-2" allele in B6 mice with a low proportion of BFU-E synthesizing DNA. The findings of Suzuki and Axelrad (10) established that Fv-2 or a closely linked gene had a significant influence on cell proliferation at a particular stage in erythrocytic differentiation. Thus it was hoped that our strategy of congenic immunization would generate a limited number of antibodies, among which might be found antibody directed against the Fv-2 gene.

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¹ Abbreviations used in this paper: BFU-E, early erythropoietic progenitor cell; C, complement; CFU-E, late erythrocytic progenitor cell; PBS, phosphate-buffered saline.
products or against putative erythrocytic differentiation antigens coded for by genes closely linked to Fv-2.

Our efforts have resulted in the production of two unique antisera, a B6-anti-B6.S and a B6.S-anti-B6 serum. Each antiserum reacts against antigen on the late erythrocytic progenitor cells CFU-E from the opposite mouse strain. These antigens, which were found to be washable from the surface of the CFU-E, were not detected on the earlier erythropoietic progenitor cells BFU-E.

Materials and Methods

Mice. B6.S strain mice (Fv-2S) were produced by crosses between C57BL/6J Ut (Fv-2rr) and SIM/Ut (Fv-2Ss) strain mice, followed by serial intercrosses and backcrosses to the C57BL/6 strain with selection for susceptibility to Friend erythroleukemia virus; they are congenic with C57BL/6 strain mice (6, 8). B6.S and B6 mice were bred and maintained in colonies of the Division of Laboratory Animal Science, University of Toronto. In all our experiments 8–12-wk-old female mice were used.

Immunization Schedule. Two immunization schedules were used. In one protocol, 10 B6 females ~8 wk old were injected with unwashed B6.S bone marrow and spleen cells; in the other, 10 8-wk-old B6.S females were injected with similar doses of unwashed B6 bone marrow and spleen cells. A constant dose of antigen, consisting of 1.0 × 10^7 unwashed bone marrow and spleen cells in a ratio of 10:1, was administered per host intraperitoneally or subcutaneously, as required. Cells to be used as antigen were collected in phosphate-buffered saline (PBS) and pooled to give the appropriate concentration of antigen per injection.

The immunization schedule was identical in both directions. The first injection consisted of a 0.2 ml vol of antigen in a 1:1 ratio with complete Freund's adjuvant (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY) administered intraperitoneally. The second injection, given 7 d later, consisted of a 0.2-ml dose of antigen in a 1:1 ratio with incomplete Freund's adjuvant (Gibco Laboratories), also administered intraperitoneally. All further immunizations were performed using a 0.6-ml dose of antigen suspended in PBS administered at four subcutaneous sites. Mice were bled 7 d after the fourth injection, and blood was pooled for each group of animals. Thereafter, we continued a schedule in which the mice were immunized ~1 wk before blood was collected. This protocol lasted ~18 mo.

Serum Collection. Blood was collected initially via orbital bleeding with a pasteur pipette and later via tail bleedings. For each group of bleeds we pooled the blood in a 10-ml plastic tube (Falcon Labware, Oxnard, CA), then allowed the blood to clot at room temperature for 2 h. The clot was removed by low speed centrifugation after retraction overnight at 4°C. Following this the serum was diluted 1:1 with minimal essential alpha medium (University of Toronto) and passed through a 0.45-µm millipore filter. Aliquots of this serum were stored at −20°C until use.

Antiserum Treatment of Cells. Bone marrow or spleen cells were collected in alpha medium. Cells were centrifuged at 160 g for 10 min and resuspended in an aliquot of cold alpha medium (washed cells) or in the original collection medium (unwashed cells). Bone marrow cells were suspended to give a final concentration of 10^7 cells per ml and 0.5 ml of this suspension (5 × 10^6 cells) was incubated in the presence of 0.25 ml antiserum and 0.25 ml Low-Tox rabbit complement (C) (Cedarlane Laboratories, Ontario), in a 10-ml sterile Falcon plastic tube. Spleen cells were diluted to give a concentration of 2 × 10^7 cells/ml and 0.5 ml of this suspension was incubated with 0.25 ml antiserum and 0.25 ml complement. All dilutions were performed with cold alpha medium. Tubes were shaken and placed on ice for 30 min and at 37°C for 20 min. During this incubation the tubes were shaken frequently. At the end of incubation the cells were washed with 5 vol of cold alpha medium and resuspended in 1.0 ml of Hanks' supplemented medium (2).

Cell Assays. The plasma culture medium used for the assay of CFU-E has been described by McLeod et al. (2) and for BFU-E by McLeod et al. (11).
### Table I

The Effect of B6 Anti-B6.S Mouse Serum on Washed and Unwashed B6.S Bone Marrow and Spleen Erythrocytic Progenitor Cells CFU-E

|                        | Mean number of erythrocytic colonies ± S.E. per 5 × 10⁴ bone marrow cells or per 10⁵ spleen cells | Percent inhibition ± S.E. |
|------------------------|--------------------------------------------------------------------------------------------------|---------------------------|
| **Bone marrow**        |                                                                                                  |                           |
| Washed cells           |                                                                                                  |                           |
| Normal B6 serum (1:8) + C (1:8) | 598.8 ± 31.9                                                                                      | —                         |
| B6.S anti-B6 serum (1:8) + C (1:8) | 536.0 ± 36.7                                                                                      | 10.5 ± 8.1                |
| B6 anti-B6.S serum (1:8) + C (1:8) | 493.3 ± 31.5                                                                                      | 17.6 ± 7.5                |
| Unwashed cells         |                                                                                                  |                           |
| Normal B6 serum (1:8) + C (1:8) | 645.0 ± 50.5                                                                                      | —                         |
| B6.S anti-B6 serum (1:8) + C (1:8) | 602.6 ± 32.5                                                                                      | 6.6 ± 9.3                 |
| B6 anti-B6.S serum (1:8) + C (1:8) | 319.7 ± 28.2                                                                                      | 50.4 ± 9.8                |
| **Spleen**             |                                                                                                  |                           |
| Washed cells           |                                                                                                  |                           |
| Normal B6 serum (1:8) + C (1:8) | 84.0 ± 3.9                                                                                       | —                         |
| B6.S anti-B6 serum (1:8) + C (1:8) | 78.5 ± 4.2                                                                                       | 6.5 ± 6.7                 |
| B6 anti-B6.S serum (1:8) + C (1:8) | 72.2 ± 3.6                                                                                       | 14.0 ± 6.3                |
| Unwashed cells         |                                                                                                  |                           |
| Normal B6 serum (1:8) + C (1:8) | 126.8 ± 7.5                                                                                       | —                         |
| B6.S anti-B6 serum (1:8) + C (1:8) | 131.8 ± 12.5                                                                                      | 0                         |
| B6 anti-B6.S serum (1:8) + C (1:8) | 69.8 ± 3.3                                                                                       | 45.0 ± 7.0                |

5 × 10⁶ B6.S bone marrow or 10⁷ B6.S spleen cells, washed or unwashed, were incubated with normal B6 serum, B6.S anti-B6 serum, or B6 anti-B6.S serum at a final dilution of 1:8. Rabbit C was also added to this mixture at a concentration of 1:8, final. Cells incubated in alpha medium (not shown) gave similar colony plating efficiencies as normal B6 serum controls. Percent inhibition was calculated as:

\[
\left( \frac{\text{Mean number of erythrocytic colonies after normal serum treatment}}{\text{Mean number of erythrocytic colonies after antiserum treatment}} \right) \times 100\%.
\]

### Results

**Effect of Congenic Antibody on Colony Formation by CFU-E in Washed and Unwashed Bone Marrow and Spleen Cell Suspensions.** When B6.S bone marrow cells were resuspended in their original collection medium (unwashed), treated with antiserum (B6-anti-B6.S), and plated in plasma culture, inhibition of erythrocytic colony formation to ~50% of control was observed (Table I). In contrast, treatment of washed B6.S bone marrow cells with the same serum resulted in only a minor reduction in number of erythrocytic colonies. Exposure of either washed or unwashed cells to normal B6 serum did not significantly inhibit erythrocytic colony formation by CFU-E in the same bone marrow cell suspensions as compared to alpha medium controls (data not shown). Antiserum raised in B6.S mice against unwashed bone marrow and spleen cells from the B6 congenic mouse strain gave an insignificant reduction in the number of erythrocytic colonies produced by washed B6.S bone marrow cells. This observation was unaltered when unwashed B6.S cells were used.
The Effect of B6.S Anti-B6 Mouse Serum on Washed and Unwashed B6
Bone Marrow and Spleen Erythrocytic Progenitor Cells CFU-E

| Bone marrow | Washed cells | Normal B6.S serum (1:8) + C (1:8) | 511.0 ± 21.6 | — |
|             |             | B6 anti-B6.S serum (1:8) + C (1:8) | 515.5 ± 41.4 | 0 |
|             |             | B6.S anti-B6 serum (1:8) + C (1:8) | 409.3 ± 37.8 | 20.6 ± 8.9 |
|             | Unwashed cells | Normal B6.S serum (1:8) + C (1:8) | 467.7 ± 15.1 | — |
|             |             | B6 anti-B6.S serum (1:8) + C (1:8) | 603.3 ± 43.5 | 0 |
|             |             | B6.S anti-B6 serum (1:8) + C (1:8) | 288.0 ± 11.1 | 52.3 ± 5.7 |

| Spleen | Washed cells | Normal B6.S serum (1:8) + C (1:8) | 34.8 ± 3.9 | — |
|        |             | B6 anti-B6.S serum (1:8) + C (1:8) | 45.3 ± 2.6 | 17.3 ± 8.6 |
|        |             | B6.S anti-B6 serum (1:8) + C (1:8) | 47.0 ± 5.0 | 14.2 ± 11.5 |
|        | Unwashed cells | Normal B6.S serum (1:8) + C (1:8) | 52.0 ± 4.5 | — |
|        |             | B6 anti-B6.S serum (1:8) + C (1:8) | 59.7 ± 6.8 | 0 |
|        |             | B6.S anti-B6 serum (1:8) + C (1:8) | 22.5 ± 4.0 | 56.7 ± 12.5 |

5 × 10⁶ bone marrow or 10⁷ B6 spleen cells, washed or unwashed, were incubated with normal B6.S serum, B6-anti-B6.S serum, or B6.S-anti-B6 serum at a final dilution of 1:8. Rabbit C was also added to this mixture at a concentration of 1:8, final. Cells incubated in alpha medium (not shown) gave similar colony-plating efficiencies as normal B6.S serum controls. Percent inhibition was calculated by the formula shown in Table I.

The action of B6 anti-B6.S serum on erythrocytic colony formation by B6.S spleen cells was also studied. We found that exposure of B6.S spleen cells to normal B6 serum did not reduce the erythrocytic colony-forming efficiency of the washed or the unwashed spleen cells when compared to alpha medium controls (data not shown). Similarly, treatment of washed or unwashed B6.S spleen cells with B6.S-anti-B6 serum showed no effect on erythrocytic colony formation by their progenitor cells. However, the B6 anti-B6.S serum produced a 45% inhibition of erythrocytic colony formation by unwashed spleen cells, but only an insignificant 14% reduction on washed cells from B6.S spleen.

We next used the same approach to determine the activity of B6.S-anti-B6 serum against erythroid progenitor cells from the B6 strain. Normal serum from B6.S mice did not affect erythrocytic colony formation by washed or unwashed B6 bone marrow or spleen cells, as compared to alpha medium controls (data not shown). In no instance did the B6-anti-B6.S serum influence erythrocytic colony formation by washed or unwashed B6 bone marrow or spleen cells. Only a minor inhibition by this antiserum of colony formation by washed spleen cells was observed (Table II).

When unwashed B6 bone marrow and spleen cells were incubated with the B6.S-
anti-B6 serum before seeding in plasma cultures, erythrocytic colony formation by bone marrow cells was reduced by 52%, and by spleen cells by 57%. Only a 21% inhibition of erythrocytic colony formation was observed when washed B6 bone marrow cells and a 14% inhibition when washed spleen cells were incubated with the same antiserum.

A titration of B6-anti-B6.S mouse serum was performed on unwashed B6.S bone marrow progenitors. Fig. 1 shows that a maximum inhibition of erythrocytic colony formation by CFU-E was observed at a serum dilution of 1:40. Activity in the serum against the colony-forming cells diminished rapidly between dilutions 1:80 and 1:160. No significant inhibition of erythropoietic burst formation by BFU-E was observed by this same antiserum at any of the dilutions tested with the exception of a questionable effect at a 1:40 dilution.

When B6.S-anti-B6 serum was titrated on unwashed B6 bone marrow cells (Fig. 2), maximal inhibition of erythrocytic colony formation was observed up to a 1:40 dilution of the antiserum. In subsequent dilutions, antibody activity diminished rapidly. This antiserum did not affect the formation of erythropoietic bursts by BFU-E.

Effect of Congenic Antibody on Burst Formation by BFU-E in Washed and Unwashed Bone Marrow and Spleen Cell Suspensions. Because the proliferative status of the BFU-E differs between the B6 and B6.S mouse strains (10), we decided to examine in more detail the effect of our antisera, raised between these congenic strains, on the burst-forming activity of BFU-E found in the bone marrow and spleens of these animals.
Fig. 2. Titration curve of the B6.S anti-B6 mouse serum inhibition of erythrocytic colony formation by CFU-E (——) and erythropoietic burst formation by BFU-E (——) of unwashed B6 bone marrow. \(5 \times 10^6\) cells were exposed to serial dilutions of the antibody, and aliquots of these cells were seeded, after washing, in plasma culture medium. The B6 anti-B6.S serum control and alpha medium control gave plating efficiencies similar to those of the normal B6.S serum control. Percent inhibition was calculated by the formula shown in Table I.

The data presented in Tables III and IV show the results of these experiments. Bone marrow and spleen BFU-E obtained from B6.S mice were unaffected by exposure to normal B6 serum as compared with alpha medium controls (data not shown). Insignificant reductions in burst formation were found when washed or unwashed B6.S bone marrow or spleen cells were treated with B6.S anti-B6 serum (Table III). Unlike the observations recorded on the reduction in number of erythrocytic colonies formed by CFU-E as a consequence of B6-anti-B6.S serum treatment, the same serum failed to produce a significant reduction in the number of erythropoietic bursts produced by BFU-E among B6.S washed or unwashed bone marrow or spleen cells (Table III).

We next attempted to assay the B6.S anti-B6 serum for activity against BFU-E from the B6 mouse strain. Results obtained in this series of experiments (Table IV) were again negative, similar to those obtained previously on B6.S erythropoietic burst formation with the anti-B6.S serum. Exposure of B6 bone marrow cells to normal B6.S serum did not affect the burst-forming efficiency of these progenitor cells as compared to alpha medium controls (data not shown). Also, the control B6-anti-B6.S serum gave an insignificant inhibition of burst formation by washed B6 bone marrow cells. The B6.S-anti-B6 serum also produced a 17% inhibition of burst formation by the erythropoietic progenitor cells which, however, did not reach the level of statistical significance. No significant inhibition of erythropoietic burst formation by B6.S-anti-B6 serum treatment of washed or unwashed B6 spleen cells was observed.

Thus, CFU-E but not BFU-E appeared to be susceptible to inhibition by antisera.
TABLE III
The Effect of B6 Anti-B6.S Mouse Serum on Washed and Unwashed B6.S Bone Marrow and Spleen Erythropoietic Progenitor Cells BFU-E

|                          | Mean number of erythropoietic bursts ± S.E. per 10^5 bone marrow or spleen cells | Percent inhibition ± S.E. |
|--------------------------|----------------------------------------------------------------------------------|---------------------------|
| Bone marrow              |                                                                                  |                           |
| Washed cells             |                                                                                  |                           |
| Normal B6 serum (1:8) + C (1:8) | 44.7 ± 3.6                                                                         | —                         |
| B6.S anti-B6 serum (1:8) + C (1:8) | 42.2 ± 2.0                                                                         | 5.6 ± 9.2                |
| B6 anti-B6.S serum (1:8) + C (1:8) | 47.0 ± 2.2                                                                         | 0                         |
| Unwashed cells           |                                                                                  |                           |
| Normal B6 serum (1:8) + C (1:8) | 44.2 ± 2.5                                                                         | —                         |
| B6.S anti-B6 serum (1:8) + C (1:8) | 47.0 ± 3.1                                                                         | 0                         |
| B6 anti-B6.S serum (1:8) + C (1:8) | 44.0 ± 2.1                                                                         | 0.5 ± 8.1                |
| Spleen                   |                                                                                  |                           |
| Washed cells             |                                                                                  |                           |
| Normal B6 serum (1:8) + C (1:8) | 8.7 ± 1.0                                                                          | —                         |
| B6.S anti-B6 serum (1:8) + C (1:8) | 8.2 ± 0.7                                                                          | 5.7 ± 14.8               |
| B6 anti-B6.S serum (1:8) + C (1:8) | 7.8 ± 0.5                                                                          | 10.3 ± 12.8              |
| Unwashed cells           |                                                                                  |                           |
| Normal B6 serum (1:8) + C (1:8) | 10.5 ± 1.6                                                                         | —                         |
| B6.S anti-B6 serum (1:8) + C (1:8) | 9.8 ± 1.5                                                                          | 6.7 ± 21.0               |
| B6 anti-B6.S serum (1:8) + C (1:8) | 8.8 ± 0.3                                                                          | 16.2 ± 15.7              |

5 × 10^5 bone marrow or 10^7 B6.S spleen cells, washed or unwashed, were incubated with normal B6 serum, B6.S-anti-B6 serum, or B6-anti-B6.S serum at a final dilution of 1:8. Rabbit C was also added to this mixture at a concentration of 1:8, final. Cells incubated in alpha medium (not shown) gave similar burst-plating efficiencies as normal B6 controls. Percent inhibition was calculated by the formula shown in Table I.

Discussion

The data presented in this study are consistent with the hypothesis that our congenic anti-B6.S and anti-B6 antisera define erythrocytic differentiation antigens at the surface of the late erythrocytic progenitor cells, CFU-E. A reduction of ~50% in number of erythrocytic colonies arising from marrow and spleen CFU-E was produced by both antisera. In none of the experiments did we demonstrate a significant antiserum-induced reduction in number of erythropoietic bursts formed by BFU-E. The antisera were specific since normal sera had no effect, and neither antiserum reacted against CFU-E obtained from the mouse strain in which the particular antiserum was generated.

A simple explanation for the failure of our antisera to show interaction with BFU-E would be that the BFU-E does not possess the antigenic material at the cell surface that binds these antibodies, and thus CFU-E and BFU-E are qualitatively different. Alternatively, the BFU-E may possess the same surface antigenic determinants but in low concentration. The plasma culture burst-inhibition assay method

produced in the congenic partner strains. Washing of these cells rendered them insusceptible to the inhibitory action of the antisera.
Table IV

The Effect of B6.S Anti-B6 Mouse Serum on Washed and Unwashed B6 Bone Marrow and Spleen Erythropoietic Progenitor Cells BFU-E

|          | Mean number of erythropoietic bursts ± S.E. per 10⁶ bone marrow or spleen cells | Percent inhibition ± S.E. |
|----------|---------------------------------------------------------------------------------|--------------------------|
| Bone marrow |                                                                                |                          |
| Washed cells | Normal B6.S serum (1:8) + C (1:8) 35.8 ± 2.4 -- | 11.1 ± 7.4 |
|             | B6 anti-B6.S serum (1:8) + C (1:8) 31.8 ± 1.2 | 16.8 ± 10.3 |
|             | B6.S anti-B6 serum (1:8) + C (1:8) 29.8 ± 2.8 |                          |
| Unwashed cells | Normal B6.S serum (1:8) + C (1:8) 31.3 ± 2.6 -- | 5.8 ± 13.8 |
|             | B6 anti-B6.S serum (1:8) + C (1:8) 32.8 ± 1.6 | 0 |
|             | B6.S anti-B6 serum (1:8) + C (1:8) 29.5 ± 3.4 |                          |
| Spleen |                                                                                |                          |
| Washed cells | Normal B6.S serum (1:8) + C (1:8) 11.8 ± 1.7 -- | 19.5 ± 15.9 |
|             | B6 anti-B6.S serum (1:8) + C (1:8) 12.7 ± 0.6 | 0 |
|             | B6.S anti-B6 serum (1:8) + C (1:8) 9.5 ± 0.8 |                          |
| Unwashed cells | Normal B6.S serum (1:8) + C (1:8) 10.2 ± 0.9 -- | 0 |
|             | B6 anti-B6.S serum (1:8) + C (1:8) 11.2 ± 0.9 | 0 |
|             | B6.S anti-B6 serum (1:8) + C (1:8) 12.2 ± 1.5 |                          |

5 × 10⁶ B6 bone marrow or 10⁷ B6 spleen cells, washed or unwashed were incubated with normal B6.S serum, B6-anti-B6.S serum, or B6.S-anti-B6 serum at a final dilution of 1:8. Rabbit C was also added to this mixture at a concentration of 1:8, final. Cells incubated in alpha medium (not shown) gave similar plating efficiencies as normal B6.S serum controls. Percent inhibition was calculated by the formula shown in Table I.

thus may simply be too insensitive to detect the antigens on BFU-E. This would imply a quantitative difference between CFU-E and BFU-E with respect to these cell surface antigenic determinants. Whether the difference turns out to be qualitative or quantitative, it is clear that expression of the antigenic determinants on the surface of erythropoietic progenitor cells varies with the stage of differentiation along the erythrocytic lineage.

Washing or not washing of the bone marrow or spleen cells was critical to the detection of antibody action against CFU-E. Among bone marrow or spleen cells collected in alpha medium, centrifuged, and resuspended in fresh alpha medium (washed), the CFU-E were unaffected by either antibody. However, when such washed bone marrow or spleen cells were resuspended in supernatants obtained from the same centrifuged bone marrow or spleen cells (unwashed), the CFU-E were found to be strongly inhibited by both antibodies. On this basis, we have postulated that the antigenic determinants recognized by both antisera are associated with the cell surface, and the fact that these antigens appear to dissociate from the cell surface as a result of simply washing with alpha medium implies that they are not firmly anchored in the cell membrane of the CFU-E. Moreover, the fact that the CFU-E can be rendered vulnerable to the antibodies by exposure to the supernatant suggests that
an entity bearing the antigenic determinants and present in these supernatants can become adsorbed to the surface of the CFU-E. It is not known whether erythrocytic colony inhibition after treatment of bone marrow and spleen cells with anti-B6 and anti-B6.S antisera occurred via a cytotoxic mechanism. The plasma culture system contains minor amounts of complement, hence antibody dependence on complement could not be assessed. Culture media free of complement will be required to resolve this problem.

The maximal inhibitory effect of 50% by our antisera is unexplained. Perhaps this indicates the existence of subpopulations within the CFU-E compartment (13, 14), or alternatively, the antigenic molecules may only interact with progenitor cells during a particular phase of their cell cycle (15). This possibility postulates the appearance, only during a specific phase of the cell cycle, of surface receptors that would render the cell susceptible to regulation by factor(s) binding to such receptors. Either explanation would account for the 50% inhibition by the congenic antisera of CFU-E present in bone marrow and spleen tissues.

The two congenic antibodies behaved identically against the CFU-E from mice of the opposite Fv-2 genotype. However, it is still unclear whether the antigenic determinants detected by these antisera are coded for by alleles at the Fv-2 locus or by different genes closely linked on the segment of chromosome 9 by which the B6 and B6.S strains differ. To our knowledge, there are no precedents in the literature for erythrocytic antigens coded for by genes in this chromosome segment. Experiments are in progress to examine these possibilities, and we are currently completing an analysis of antibodies produced by hybridomas constructed from mice used to raise the two antisera described here.

Overall, the results obtained indicate that novel erythrocytic differentiation antigens at the CFU-E level have been identified by the antisera we have produced. Both antisera display immunologic, genetic, and cellular specificity, but functions of the antigens they recognize are unknown. A number of studies (16, 17) have shown that shed cell surface molecules may function as regulators of growth and differentiation in a variety of experimental systems. It is thus possible that the B6 and B6.S antigens detected in this study may also function as regulators, in this case along the erythrocytic lineage. This possibility is presently under investigation in our laboratory.

**Summary**

We have investigated the activities of alloantisera produced in B6 (C57BL/6) and B6.S strain mice reciprocally immunized with unwashed bone marrow and spleen cell suspensions from their respective Fv-2 congenic partner strains, B6.S and B6. These antisera inhibited the formation of colonies by the late erythrocytic progenitors (CFU-E) in plasma cultures seeded with unwashed bone marrow or spleen cells; washed cells were unaffected. Erythropoietic burst formation by the early progenitors (BFU-E) was not significantly inhibited by the antisera, whether the cells were washed or unwashed. We conclude (a) that the congenic antisera are capable of recognizing alloantigens controlled by alleles of Fv-2 or of a closely linked gene locus on chromosome 9; (b) that these alloantigens are situated on the surface of erythrocytic progenitor cells and can be removed by washing; and (c) that the expression of the alloantigens on these cells is influenced by their stage of differentiation.

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