BRAIN-ASSOCIATED STEM CELL ANTIGEN: AN ANTIGEN SHARED BY BRAIN AND HEMOPOIETIC STEM CELLS*

BY EDWARD S. GOLUB†

(From the Department of Biological Sciences, Purdue University, Lafayette, Indiana 47907)

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There is evidence that pluripotent stem cells found in fetal liver and adult bone marrow of the mouse can repopulate the myeloid and lymphoid tissues of irradiated mice (1-4). This evidence has been accumulated in part by examining the histological and functional characteristics of spleen colonies obtained by the method of Till and McCulloch (5). Each spleen colony in this assay is the progeny of a single colony-forming unit (cfu) or stem cell. Thus, a pluripotent cell in the bone marrow has the potential to differentiate along a variety of pathways depending upon the inductive stimulus which it receives. The problem of the mechanism and control of differentiation of the hemopoietic stem cell to the various fully differentiated stages is of great importance in several areas of research.

In this communication we report the production of an antibody to the colony-forming unit. The source of antigen was mouse brain. It is known that mouse brain has an antigen cross-reactive with the θ antigen on thymocytes and thymus-derived cells (T cells) (6). We have previously shown (7) that immunization of rabbits or goats with mouse brain resulted in antisera which had the properties of anti-θ antisera. These antisera were cytotoxic for mouse thymocytes but not for bone marrow cells, did not reduce the number of plaque-forming cells in an immune response (thus demonstrating that they did not have anti-B cell activity), and prevented a graft-versus-host reaction and the induction of a primary immune response in vitro. We have now tested these antisera for the ability to prevent the formation of hemopoietic colonies (anti-cfu activity) and have found an apparent anti-stem cell antibody.

Materials and Methods

Animals.—CBA, C57BL/6, and BDF1 [(C57BL/6 × DBA/2)F1] mice were bred in our colony using stocks obtained from Jackson Laboratory, Bar Harbor, Maine. BALB/cJ mice were obtained from Jackson Laboratory. Animals were maintained five to a cage with drinking water containing 11 ppm chlorine at pH 2.8 (8).

Anti-Brain Antisera.—The method previously described (7, 9) was used. Mouse brain was homogenized in a Sorval Omnimixer (Ivan Sorvall, Inc., Norwalk, Conn.) and emulsified in...
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Freund's complete adjuvant (Difco Laboratories, Inc., Detroit, Mich.). Rabbits or goats were injected with 3 ml of the mixture and bled at suitable intervals after secondary injections.

Colony-Forming Unit Assay.—The method of Till and McCulloch was used (5). Groups of mice were X-irradiated using a G.E. Maxitron machine (General Electric Co., Pleasanton, Calif.). The conditions of irradiation were 250 kv, 15 ma, 2.5 mm copper, and 1 mm aluminum filtration, head height 51 cm, 50 R/min. The total dose was 800 R delivered in a split dose of 400 R on each of 2 days. Suitable numbers of normal syngeneic bone marrow cells were obtained from femurs and tibias, washed, and injected intravenously. After 10 days the mice were sacrificed, the spleens placed in Bouin's solution, and the number of colonies counted.

Bone marrow cells treated with antiserum were treated as follows: 1 X 10^7 bone marrow cells in 0.1 ml buffer (7) were reacted with 0.1 ml of a 1/3 dilution of the antiserum. The antiserum was absorbed before use with erythrocytes of the strain of mouse being used. After 30 min in the cold, guinea pig complement (1/5) (Hyland Div., Travenol Laboratories, Inc., Costa Mesa, Calif.) was added and incubated at 37°C for 1 hr.

RESULTS

Effect of Anti-Brain Antisera on Colony-Forming Units.—Three preparations of anti-mouse brain antisera were tested for their ability to inhibit colony formation. Goat anti-CBA brain, goat anti-BDF1 brain (kindly provided by Dr. R. W. Dutton), and rabbit anti-BALB/c brain were tested. Normal goat and normal rabbit sera were used as controls. Bone marrow from CBA, C57BL/6, and BDF1 mice were tested, as was fetal liver from CBA. After treatment of the cells as described in Materials and Methods, appropriate numbers of cells were injected into lethally irradiated syngeneic mice. 6 X 10^4 C57BL/6 and BDF1 bone marrow cells or 1 X 10^6 CBA bone marrow cells; 10^6 CBA fetal liver cells injected into lethally irradiated syngeneic mice.

Effect of Absorption of Antiserum on Colony-Forming Unit Inhibition and Anti-Thymocyte Cytotoxicity.—These antisera showed no cytotoxicity for bone marrow cells (7) as measured by trypan blue exclusion. However, the antiseras

| Treatment of bone marrow  | Bone marrow cells*  | Fetal liver cells* |
|--------------------------|---------------------|-------------------|
|                          | CBA                 | C57BL/6           | BDF1              | CBA   |
| Goat anti-CBA brain      | 0.7 ± 0.6f          | 1.1 ± 0.9         | 0.9 ± 0.3         | 0.4 ± 0.1 |
| Goat anti-BDF1 brain     | -f                  | -                 | 1.2 ± 0.3         | -     |
| Normal goat serum        | 10 ± 2.3            | 11 ± 1.4          | 15 ± 1.2          | 15 ± 1.2 |
| Rabbit anti-BALB/c brain| 0.8 ± 0.2           | -                 | -                 | -     |
| Normal rabbit serum      | 17 ± 1.9            | -                 | -                 | -     |

* 6 X 10^4 C57BL/6 and BDF1 bone marrow cells or 1 X 10^6 CBA bone marrow cells; 10^6 CBA fetal liver cells injected into lethally irradiated syngeneic mice.

† Colonies at day 10 ± standard error.
§ From Dr. R. W. Dutton.
¶ Not done.
had high titers of anti-thymocyte activity. It was important to determine if the two activities of the sera could be separated by absorption. Accordingly, samples of the anti-brain antisera were absorbed with brain, thymus, or liver and the absorbed antisera tested for both inhibition of colony-forming unit activity and anti-thymocyte cytotoxic activity. The results of these experiments are seen in Table II. It is clear from the data in the table that absorption with brain or thymus removed all of the anti-thymus cytotoxic activity of the antisera. Absorption with liver did not reduce the potency of the antisera against thymus cells or cfu. The anti-stem cell activity of the antiserum was removed only after absorption with brain and not after absorption with thymus. Thus the two activities of the anti-brain antisera could be separated by absorption with thymocytes.

Absorption of the antisera with bone marrow did not reduce the anti-cfu

| Goat anti-CBA brain* absorbed with | Colonies/10⁶ CBA bone marrow | Anti-thymus % killing (corrected for background)§ |
|-----------------------------------|-------------------------------|-----------------------------------------------|
| CBA brain                         | 12.0 ± 1.8                    | 9                                             |
| BDF1 brain                        | 11.4 ± 1.6                    | 9                                             |
| CBA thymus                        | 1.1 ± 0.5                     | 7                                             |
| CBA liver                         | 3.8 ± 1.3                     | 100                                           |
| Rat brain                         | 4.2 ± 1.1                     | 7                                             |
| Human brain                       | 2.6 ± 1.3                     | 5                                             |
| Normal goat serum (control)       | 13.1 ± 1.5                    | --                                            |

* 1 g brain tissue was shredded and washed and used to absorb 1 ml of mouse RBC-absorbed goat anti-mouse brain antiserum. Final dilution of the serum was 1/12.

§ % killing (corrected for background): \( \frac{\% \text{ experimental dead} - \% \text{ control dead}}{100 - \% \text{ control dead}} \times 100. \)

TABLE III
Effect of Various Anti-Brain Antisera on the Ability of Mouse Bone Marrow to Form Colonies

| Goat anti-CBA brain               | 1 ± 0.3                        |
| Rabbit anti-BALB/c brain          | 0.8 ± 0.2                      |
| Rabbit anti-rat brain             | 11 ± 1.7                       |
| Rabbit anti-human brain           | 20 ± 1.3                       |
| Rabbit anti-dog brain             | 12 ± 1.3                       |
| Normal goat serum                 | 18 ± 2.0                       |
| Normal rabbit serum               | 17 ± 1.9                       |

* 1 × 10⁶ treated CBA bone marrow cells injected into 800 R CBA recipients. Colonies at day 10 ± standard error.
activity of the antisera (data not shown) but this is probably due to the fact that the cell with the relevant antigen, the stem cell, is in such low concentration in the bone marrow (see Table I). It is of interest that only mouse brain absorbed anti-cfu activity. This is in contrast to our other findings (9) that the brains of most species contain an antigen which cross-reacts with the mouse brain-associated thymus antigen (BAθ). We tested the possibility that we were missing the absorption of the anti-stem cell antibody by brain tissue of other species by testing higher dilutions of antiserum absorbed with rat brain. Even at the ends of the titration curve we found no inhibition of cfu.

**Effect of Anti-Brain Antiserum to Other Species on Mouse Colony-Forming Units.**—To further test the question of sharing of cross-reactive brain-associated stem cell antigen between the mouse and other species, antisera against rat, dog, and human brain were tested on mouse bone marrow cells. The results of these experiments are seen in Table III. Only the anti-mouse brain antiserum had activity against the mouse stem cell.

**DISCUSSION**

The data in the present study show that there is an antigen on brain which is shared by the hemopoietic colony-forming unit or stem cell. Neither liver nor thymus shares this antigen. The brain also has an antigen which it shares with thymocytes (θ) (6) and antisera against brain are active against thymocytes (7). Absorption of the anti-brain antiserum with thymocytes removes anti-thymus activity (7, 9) but does not remove anti-stem cell activity.

The brain also shares an antigen with liver and lymphoma cells (R. Hyman and E. S. Golub, unpublished data). Anti-brain antiserum are cytotoxic for both θ+ and θ− lymphomas. Absorption of the antiserum with thymus only does not remove the cytotoxic effect for either. Absorption with liver only removes cytotoxicity for θ− lymphomas but not for θ+ lymphomas. This probably indicates an antibody with another specificity in the antiserum. We are currently testing the effect of these antisera on other tumors and also the effect of the antiserum on the lymphomas after absorption with other tumors. Antiserum against mouse brain also contains an antibody against mouse erythrocytes. The antibody raised against CBA or BALB/c brain, for example, reacts with the erythrocytes of eight different strains and seems to be independent of any known histocompatibility antigen.1

1 Golub, E. S. Brain associated erythrocyte antigen (BAeryth): an antigen shared by brain and erythrocytes. Manuscript in preparation.

It is also known that brain contains the PC1 antigen which is shared by plasma cells (10). The anti-stem cell antibody is different from anti-PC1 antibody which could be obtained from PC1 positive strains (CBA is PC1 negative) because the anti-brain sera used in this study have no anti-plaque-forming
cell activity. Nor do the sera have anti-precursor activity since treated bone marrow cells are able to cooperate with untreated thymus cells in an irradiated host (Cudkowicz and Miller, personal communication).

The anti-mouse brain antiserum has been shown to inhibit cfu in vitro (J. Watson and E. S. Golub, unpublished data) and there is a possibility that anti-human brain antiserum has anti-human cfu activity. We are testing this possibility in vitro. The experiments here also show that each of the strains of mice tested has the antigen on a subpopulation of cells in its bone marrow. If there are alleles of this stem cell antigen as there are with \( \theta \) (4) the heterologous species (goat or rabbit) may not be able to detect a difference. This is the case with brain-associated \( \theta \) (6). Van Bekkum and his coworkers have been able to concentrate the colony-forming cell by gradient centrifugation (11). Hopefully a combination of these enrichment techniques and the anti-cfu antibody will provide a powerful tool for the study of the stem cell.

One can only speculate upon the significance of the fact that the brain of the mouse has at least four and possibly five identified antigens common to lymphoid tissue. At least two of these antigens, \( \theta \) and PC1, are "developmental antigens" (12). As other antigens are characterized it will be of interest to determine if the brain, with its blood-brain barrier sequestered antigens, is a reservoir of the potential antigens which stem cells can express at various stages of differentiation along the myeloid and lymphoid lines. The fact that an antibody apparently against the cfu is now available, in any case, provides a potent tool for elucidating the differentiative pathways of the stem cell.

**SUMMARY**

There is an antigen on mouse brain tissue which is shared by the hemopoietic colony-forming unit or stem cell of the mouse. Treatment of bone marrow or fetal liver cells with anti-brain antisera inhibits expression of colony-forming units. The anti-stem cell antibody is not absorbed by thymus cells and thus can be distinguished from the anti-thymocyte antibody which these antisera also contain.

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