

Brief Definitive Reports

ANTIGENIC DIFFERENCES BETWEEN HEMOPOIETIC STEM CELLS AND MYELOID PROGENITORS*

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There is general agreement that the mature cells of the blood of mammals arise from a pluripotent hemopoietic stem cell (1-4). This cell is defined by ability to form spleen colonies after injection into lethally irradiated mice (5) and is thus called a spleen colony-forming unit or CFU-s. Colonies of myeloid cells can be formed in vitro (6, 3) and the cells which give rise to these colonies (CFU-c) differ in many properties from the CFU-s. The CFU-c appear to be slightly denser (8, 9), have a higher sedimentation rate, (8) and have a larger proportion of cells in S phase of the cell cycle than CFU-s (10, 11). It seems reasonable to consider the CFU-c as a different, more differentiated cell than the CFU-s which may be considered the earliest known hemopoietic cell. The cell responsive to the inductive factors which either cause or allow expression along a given differentiative pathway is called the progenitor cell. It is not clear how far removed from the stem cell the progenitor cell is in differentiative steps or at what stage the progenitor becomes a determined cell. Once the progenitor cell has begun to differentiate along a given pathway toward an end cell the intermediate cell types may be considered precursor cells of that end cell.

An antigen shared with CFU-s was found in mouse brain making it possible to produce an anti-CFU-s (or an antistem cell) antibody (12). This brain-associated stem cell specificity was shown to be different from brain-associated θ (13) and brain-associated erythroid specificities (14). Using rabbit or goat antibrain antisera it has been possible to eliminate CFU-s from bone marrow or spleen. In this paper, we have used antistem cell antiserum to show that the progenitor cell which is responsible for in vitro myeloid colonies does not share the brain-associated stem cell antigen with the pluripotent cell responsible for spleen colonies. Thus, a clear antigenic difference can be demonstrated to have occurred in early hemopoietic differentiation.

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Materials and Methods

Animals.—BDF₁ (C57BL/6 × DBA/2) mice were used for all but one of the experiments. These mice were bred at Purdue using stocks obtained from Jackson Laboratories, Bar Harbor, Maine. Animals were maintained five to a cage with drinking water containing 11 ppm chlorine at pH 2.8 (15). CBA mice were used in the double cell transfer experiment which was carried out at Rijswijk. These animals are also maintained on low pH, high chlorine water.

X Irradiation.—The conditions for X irradiation at Purdue were as described in (12) and for Rijswijk as in (16).

Colony-Forming Unit Assays.—

CFU-s: The spleen assay of Till and McCulloch was used (5). Briefly, 5×10^4 bone marrow cells were injected i.v. into lethally irradiated mice. 10 days later the number of spleen colonies were counted.

CFU-c: Myeloid precursor cells were assayed essentially by the method described by Metcalf and Moore (17). 1×10^5 bone marrow cells were suspended in Dulbecco's medium with 0.3% agar. 10% colony-stimulating factor (CSF) extracted from mouse uteri according to the method of Bradley (18) was added and 1 ml aliquots were distributed in 35 mm Falcon dishes and incubated at 37°C with 10% CO₂ in 100% humidity. Colonies were counted at day 7. The CSF levels used were those which allowed maximal expression of CFU-c.¹

Antisera: Antistern cell antiserum was produced as described previously (12, 13). The batch used in the experiments reported here was from a goat which had received two injections of CBA brain emulsified in complete Freund's adjuvant (13). The standard antiserum was absorbed with mouse erythrocyte and liver (19). In most experiments the serum was also absorbed with thymus cells. The rabbit antigoat gamma globulin was kindly provided by Dr. R. M. E. Parkhouse, Medical Research Council Laboratory, Mill Hill, London.

RESULTS

Effect of Antimouse Brain Antiserum on In Vivo and In Vitro Colony Formation.—To determine the effects of the antistern cell antiserum on in vitro colony formation (CFU-c) and in vivo colony formation (CFU-s), bone marrow cells from BDF₁ mice were treated with dilutions of goat antimouse brain antiserum absorbed with red cells, liver, and thymus as described in Materials and Methods. After this treatment, varying concentrations of cells were injected into lethally irradiated BDF₁ recipients to determine the numbers of surviving CFU-s or cultured in vitro with CSF to determine the number of surviving CFU-c. In vitro colonies were counted at day 7 and in vivo colonies at day 10. The results of three such experiments are presented in Table I.

It can be seen that there was a 75–85% reduction of CFU-s but that the number of CFU-c was not significantly reduced. This result was also obtained with three separate batches of rabbit anti-CBA brain and another batch of goat anti-CBA serum.

Evidence that the Lack of Reduction of CFU-c is not due to Inefficient In Vitro Cytotoxicity of CFU-c.—The results in the previous section can be interpreted as showing that the CFU-c does not share an antigen with CFU-s. However, it is necessary to show that the difference is not due to the fact that there is

¹ van den Engh, G. J. Quantitative in vitro studies on stimulation of murine haemopoietic cells by colony-stimulating factor. Manuscript in preparation.

TABLE I
*Recovery of CFU-c and CFU-s from Bone Marrow Cells Treated with Antibrain Antiserum**

Exp. no.	Bone marrow cells treated with:		In vitro colonies counted (av)	In vivo colonies counted (av)
1†	NGS	1/4	30, 36, 31, 39 (34)	Not done
	Antibrain ^a	1/4	55, 41, 48, 49 (48)	
	Antibrain ^b	1/4	44, 55, 64, 63 (52)	
2§	NGS	1/8	60, 47, 56 (55)	25, 28, 31, 24, 26 (27)
	Antibrain	1/4	42, 48, 46 (45)	5, 1, 3, 6, 4 (3.8)
		1/8	49, 55, 52 (52)	7, 6, 15, 8, 7 (8.6)
		1/16	42, 41, 44 (42)	7, 14, 9, 7, 9 (9.2)
		1/32	37, 38, 49 (41)	9, 19, 19, 15, 13 (15)
		1/64	40, 55, 51 (49)	27, 14, 30, 26, 18 (23)
3	NGS	1/8	78, 100 (89)	22, 14, 26, 17 (22)
	Antibrain	1/4	92, 78 (85)	4, 4, 4, 3, 12 (5.4)
		1/8	84, 70 (77)	12, 18, 8, 16 (11)
		1/16	82, 79 (80)	2, 7, 8, 6 (5.8)
		1/32	104, 72 (88)	10, 10, 16, 4 (10)
		1/64	85, 110 (97)	9, 17, 19, 12 (14.2)

* 2×10^6 BDF₁ bone marrow cells in 0.2 ml were treated with an equal volume of goat anti-CBA brain antiserum absorbed as indicated and diluted in BSS. After 30 min at OC, 5 ml guinea pig complement diluted 1/5 in BSS was added and the cells incubated for 1 h at 37C. The indicated number of cells were then injected into lethally irradiated BDF₁ mice for CFU-s assay or plated for CFU-c assay. CFU-s were assayed on day 10 and CFU-c on day 5.

† 1×10^5 cells plated. Antiserum was absorbed with a) mouse RBC and b) mouse RBC and thymus.

§ 1×10^5 cells plated and 2×10^5 cells injected for CFU-s. Antiserum was absorbed with RBC, thymus, and liver.

|| 2×10^5 cells plated for CFU-c assay and 8×10^4 cells injected for CFU-s assay. Antiserum absorbed with RBC.

a differential ability of cells to be lysed in vivo and in vitro. For example, both CFU-c and CFU-s could share the stem cell antigen and both could react with the antistem cell antibody but treatment with complement in vitro may not cause the cells to lyse. When the cells with their reacted antibody are injected into the mouse, conditions in vivo could allow lysis. The cell being tested for in vitro colony formation would not be subjected to this in vivo action and would thus express itself as a colony-forming unit even though it shared the antigen. Indeed, CFU-s which have been reacted with antistem cell antibody in vitro in the absence of complement are inhibited from forming colonies in vivo. Presumably, this is due to some action of the recipient on the cell-antibody complex in vivo. Several experiments were carried out to decide between these alternatives.

Effect of Antiglobulin Treatment.—Bone marrow cells were treated with the goat antibrain antiserum for 30 min in the cold, washed, and then reacted

with a 1/10 dilution of rabbit antigoat gamma globulin. After a further 30 min in the cold the cells were washed and treated with guinea pig complement for 1 h at 37°C and then tested for ability to form in vitro colonies. Treatment with the antiglobulin antiserum did not cause any reduction in CFU-c expression.

The Effect of In Vivo Passage of In Vitro-Treated Cells.—Bone marrow cells were treated with complement alone (Group A) or with antibrain plus complement (Group B). 10^6 of these cells were then injected into lethally irradiated mice. One group of irradiated mice received no bone marrow cells (Group C). 24 h later, the spleens of these irradiated mice were harvested, cultured in vitro to determine the number of CFU-c or injected into another set of irradiated mice to determine the number of surviving CFU-s. Thus, if the host mouse were contributing to the lysis of the antistem cell antibody-treated cells, there should be a reduction in the number of colony-forming units in the spleens of these adoptive hosts when assayed for CFU-c. If colonies were obtained from these irradiated hosts, i.e., if there were no reduction by passage through the animal, this would argue against inefficient lysis in vitro being responsible for lack of reduction of CFU-c seen after in vitro treatment.

The results of this last experiment are presented in Table II. It can be seen that there was no reduction in the number of in vitro colonies formed after passage of the cells through the irradiated host.

DISCUSSION

The experiments in this paper were designed to answer the question of whether the progenitor cell which is responsible for in vitro colonies shares the

TABLE II
*CFU Content of Spleens of Irradiated Hosts after Injection of Anti-CBA Brain-Treated Bone Marrow**

Treatment of bone marrow cells before passage through primary hosts	CFU recovered from primary hosts	
	CFU-c/spleen	CFU-s/spleen
A. Complement‡	65	9¶
B. Goat anti-CBA brain§ + complement	120	2
C. No cells	5	0

* Bone marrow cells were treated as in Table I.

‡ Responses obtained from spleens of irradiated primary hosts repopulated with 10^6 bone marrow cells treated with normal serum and complement.

§ Response obtained from spleens of irradiated primary hosts repopulated with 10^6 bone marrow cells treated with anti-CBA brain plus complement.

|| Response obtained from the spleens of irradiated mice with no repopulation of bone marrow.

¶ The original marrow suspension contained approximately 130 CFU-s/ 10^6 cells. The seeding efficiency for these cells after 24 h is approximately 10%. Thus the theoretical number of recoverable CFU-s from the primary hosts is 13.

brain-associated stem cell antigen with the cell responsible for spleen colonies. This was accomplished by determining if *in vitro* colonies were prevented from being formed after reaction of bone marrow cells with antibrain antiserum and complement. Antibrain sera contain antibodies which prevent the formation of *in vivo* colonies (12), i.e., antistem cell antibodies. The experiments reported here show that treatment of bone marrow cells with such an antiserum and complement does not prevent *in vitro* colonies from forming. It can be seen in Table I that while spleen colony formation is essentially abolished, *in vitro* colony formation is essentially unaffected. Furthermore, the same result was obtained with antisera absorbed with thymus and thus devoid of the brain-associated θ specificity (13).

The failure to impair expression of *in vitro* colonies after anti-brain antiserum treatment was shown not to be due to inefficient *in vitro* cytotoxicity. Since bone marrow cells which were reacted with antibody *in vitro*, passaged through an irradiated animal and recovered still were able to form *in vitro* colonies (Table II).

There are a number of physical and physiological differences between CFU-c and CFU-s (8-11), the most striking being that the CFU-s is pluripotent but the CFU-c is a determined cell. The CFU-c is responsive to inductive influences and microenvironments which the stem cell is not. The data in this paper show that along with these functional changes, there is an antigenic change which has also occurred in the differentiation from stem cell to progenitor cell.

We have, as of yet, no marker antigen for the progenitor cell as we do for the stem cell (12), and for several later stages of differentiation of the progenitor cell. The cells which are derived from the lymphoid progenitor and which are determined to become T lymphocytes (20) under thymic influence will express a variety of antigens at different stages of differentiation. An example of these differentiation antigens (21) on the lymphocyte in the thymus (T_0 cell) is the expression of TL and θ . After further differentiation and the export to the peripheral lymphoid tissue, these cells become T_1 and T_2 (22) thymic-derived lymphocytes and no longer express TL while still expressing θ . Similarly, we have demonstrated that B-lymphocyte precursor cells in the bone marrow and B cells in the peripheral lymphoid compartments which are responsive to the same antigen in thymic-dependent or in thymic-independent forms have characteristic surface antigens associated with their stage of development.²

It is probable that antigenic differences are associated with all or most stages in hemopoietic differentiation. If this is the case, as we identify the antigens we will have powerful tools for the study of hemopoiesis, both normal and leukemic, since we will be able to identify or eliminate cells at specific stages of differentiation. We are currently determining if the erythroid and

² Golub, E. S., M. Bradshaw, I. F. C. McKenzie, and D. Sachs. Manuscript in preparation.

lymphoid progenitor cells also lack the stem cell antigen and are attempting to identify early lymphoid precursor cells by unique surface antigens.

SUMMARY

Bone marrow contains pluripotent stem cells which give rise to colonies when injected into irradiated syngenic hosts as well as more differentiated progenitor cells of the myeloid cell which are able to form colonies in vitro. Antisera against brain is known to contain antistem cell antibody. The present experiments were designed to determine if the myeloid progenitor cell still expresses the stem cell antigen.

Bone marrow cells were treated with antibrain antiserum plus complement and then survival of stem cells was determined by injection into irradiated hosts. Survival of myeloid progenitor cells was determined by culturing the cells in vitro. It was found that stem cells were eliminated by the antiserum but that myeloid progenitors were not. Inefficient in vitro lysis was ruled out as the reason for this difference since in vitro colonies were not reduced when the cells were treated with anti-immunoglobulin or after passage through an irradiated host. In the differentiation from stem cell to myeloid progenitor there is an associated surface antigen change.

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