DEMONSTRATION OF THY-1 ANTIGEN ON PLURIPOTENT HEMOPOIETIC STEM CELLS IN THE RAT*

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Thy-1 is a cell surface antigen that is present in large amounts on thymocytes and brain of rats and mice, and at reduced or as yet undetermined levels on some peripheral lymphoid cell populations, fibroblasts, epidermal cells, and mammary tissue (1-7). Two allelic forms of the molecule, designated Thy-1.1 and Thy-1.2 (formerly θ-AKR and θ-C_3H) are found in the mouse (1). Only Thy-1.1 has been identified thus far in the rat (2). In addition to sharing this alloantigenic determinant, Thy-1 antigens in the mouse and rat share a xenoantigenic determinant recognized by rabbit antiserum (8, 9). Thy-1 antigen has been purified from rat thymocytes and brain, and has been shown for both tissues to be a glycoprotein of mol wt approximately 25,000 daltons (10, 11). Thy-1 antigen from mouse thymocytes appears to be a glycoprotein of similar size (11-14).

Despite these chemical and antigenic similarities, and the broad similarity in tissue distribution, there are important differences between the rat and mouse with respect to the distribution of Thy-1 antigen among specific lymphohemopoietic cell populations. Thy-1 is a general marker for T lymphocytes in the mouse (15), but is not readily detectable on nucleated bone marrow cells (16). Conversely, Thy-1 is present on a minority of T cells in the rat (5), but is readily detectable on as many as 45% of nucleated bone marrow cells (6). Among these cells are some members of the B lymphocyte and myeloid cell series (17, 18). Attempts to abrogate mouse thymocyte progenitor cell and hemopoietic stem cell activity with anti-Thy-1 alloantisera have been unsuccessful (16, 19-21), although recent reports indicate that a Thy-1-positive regulatory cell can enhance the proliferative capacity of mouse hemopoietic stem cells (22, 23). In contrast, anti-Thy-1 treatment of rat bone marrow cells has been found to prevent regeneration of granulocytes in a rat-into-mouse adoptive transfer system (21); and Thy-1-positive rat bone marrow cells, isolated on the fluorescence-activated cell sorter, have been shown to generate B lymphocytes in irradiated recipients (17).

These observations suggest that Thy-1 antigen in the rat may be present on

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hemopoietic stem cells and/or on the committed progenitors of the lymphoid, myeloid, or erythroid cell series. Using an in vivo spleen colony-forming unit assay (CFU-S)[1] (24, 25) and highly specific anti-Thy-1 sera, we demonstrate in the present paper that Thy-1 antigen is present on pluripotent stem cells in rat hemopoietic tissues at three developmental stages, viz. adult bone marrow, neonatal spleen, and fetal liver. Evidence that Thy-1 antigen is present on hemopoietic progenitor cells is the subject of a separate report.[2]

Materials and Methods

Animals. Fetal, newborn, and young adult (8–10 wk old) male and female Lewis strain rats and members of the (Lewis × DA)F_1 hybrid cross were used as donors of bone marrow and lymphoid cells in experiments involving transfer of antibody-treated cells (see below). Time of gestation for rat fetuses was determined by vaginal smears (appearance of sperm = day 0). Breeding stocks of these animals were maintained at the University of Connecticut Health Center. 4- to 5-wk-old male and female Lewis and (Lewis × DA)F_1 hybrid strain rats were used as recipients. The recipients received 800 rads whole body X-irradiation 2 h before they were given cells (26).

Young adult (7–9 wk old) male and female DA, Lewis, and Wistar strain rats were used as donors of bone marrow cells in experiments involving transfer of cells separated on the fluorescence-activated cell sorter (see below). Breeding stocks of these animals were maintained at the Walter and Eliza Hall Institute of Medical Research. 5- to 7-wk-old syngeneic rats were used as recipients. The recipients received 750 rads whole body X-irradiation 6 h before they were given cells. The irradiation was delivered by a Phillips RT 250 machine operating at 250 kV under maximum back scatter conditions; half value layer was 0.2 mm copper and midline dose rate was 137 rads/min.

All irradiated rats were maintained on oxytetracycline, 1.0 mg/ml drinking water.

4- to 6-wk-old male and female AKR/Jax and AKR/Cum strain mice were obtained from The Jackson Laboratory (Bar Harbor, Maine) and Cumberland Farms (Clinton, Tenn.), respectively.

Preparation of Cell Suspensions. Thymocytes, lymph node cells, spleen cells, bone marrow cells, and thoracic duct cells were collected as described previously (26, 27). Blood lymphocytes were isolated by the method of Wilson (28). Lymphocyte-rich fractions of spleen and bone marrow cell suspensions were prepared by isopyknic centrifugation on a 6.34% Ficoll-10.0% Hypaque gradient (29). Further enrichment of lymphocyte-like null cells from bone marrow was effected by passage of the lymphocyte-rich fraction through a column of fine glass beads as described previously (27). These cells have been shown to bear the bone marrow lymphocyte antigen (BMLA), but to lack antigens specific for peripheral T and B cells (27, 30).

Preparation of Thy-1 Antigen from Rat Brain. The method of Barclay et al. (10) was used with some modifications as described elsewhere.[3] Thy-1.1 antigenic activity was assayed according to the method of Williams et al. (31).

Antisera. Heteroantisera to the rat bone marrow lymphocyte antigen (anti-BMLA serum) and to rat T lymphocytes (anti-T-cell serum) were prepared in rabbits according to published methods (25, 29).

Two murine anti-Thy-1.1 alloantisera were used. The first, raised in AKR/cum mice against AKR/J mouse thymocytes, was the gift of Dr. R. T. Acton (University of Alabama); the second, a CBA mouse anti-AKR mouse thymocyte antiserum, was the gift of Dr. A. F. Williams (Oxford University).

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[1] *Abbreviations used in this paper:* BMLA, bone marrow lymphocyte antigen; CFU-S, spleen colony-forming unit (used synonymously with pluripotent hemopoietic stem cell); FACS, fluorescent-activated cell sorter; FITC, fluorescein-conjugated; null, lacking T- and B-cell antigenic markers; RITC, tetramethyl rhodamine conjugated; TdT, terminal deoxynucleotidyl transferase.

[2] I. Goldschneider et al. Demonstration of Thy-1 antigen on hemopoietic progenitor cells in rat bone marrow. Manuscript submitted for publication.

[3] R. J. Morris et al. Control of the expression of the Thy-1 cell surface glycoprotein on nerve cell lines of the rat and mouse. Manuscript submitted for publication.

The preparation and absorption of the rabbit antiserum to the Thy-1 molecule purified from rat brain is described in full elsewhere.[3] Of the anti-Thy-1 antibodies in this serum, 51% recognized the rat-specific xenoantigenic determinant, 41% recognized the rat-mouse cross-reacting xenoantigenic determinant, and approximately 8% recognized the Thy-1.1 alloantigenic determinant (assayed as in [8]). The IgG fraction from this serum was prepared by Na_2SO_4 precipitations, DEAE-Sephadex ion exchange chromatography, and Sephadex G-200 gel filtration following standard procedures. The $F(ab')_2$ fragments prepared from this (32) were conjugated with fluorescein isothiocyanate (Baltimore Biological Laboratories, Cockeysville, Md.) as described by The and Feltkamp (33). Antibodies with a fluorescein/protein molar ratio of between 1.0 and 3.4 were pooled, giving a mean fluorescein/protein ratio of 1.7 for the preparation. This rabbit anti-Thy-1 antiserum is referred to as serum number 1 in the text.

The $F(ab')_2$ of a second rabbit antiserum to rat brain Thy-1 was used for fluorescence microscopy. This serum, the gift of Dr. A. F. Williams (Oxford University), was absorbed for 1 h at 4°C with 3×10^9 rat spleen cells and 6×10^8 rat lymph node cells per ml of antiserum. The IgG fraction was isolated, from which the $F(ab')_2$ was prepared. Of its anti-Thy-1 antibodies, 45% recognize the rat-specific Thy-1 xenoantigenic determinant, 37% recognize the rat-mouse cross-reacting xenoantigenic determinant, and 18% recognize the Thy-1.1 alloantigenic determinant (10). This rabbit anti-Thy-1 antiserum is referred to as serum 2 in the text.

Rabbit $F(ab')_2$ anti-mouse-IgG purified antibodies, and horse $F(ab')_2$ anti-rabbit IgG purified antibodies, were the gift of Dr. A. F. Williams (Oxford University). Fluorescein-conjugated (FITC) goat anti-rabbit IgG and tetramethyl-rhodamine conjugated (RITC) goat anti-mouse IgG were obtained from Cappel Laboratories, Inc., Cochranville, Pa.). The latter antiserum was passed down a normal rat serum Sepharose 4B affinity column (serum at 10 mg/ml coupled to cyanogen bromide-activated Sepharose 4B) (Pharmacia Fine Chemicals, N.J.) to remove cross-reacting antibodies to rat immunoglobulins.

Protein determinations were done by the method of Lowry et al. (34) using bovine serum albumin (Sigma Chemical Co., St. Louis, Mo., Fraction V) as a standard.

Quantitative Absorptions of Antisera. Antisera were absorbed with deoxycholate-free purified Thy-1 by incubating (at 4°C) twofold serial dilutions of the antigen (in 125 μl of Tyrode's buffer with a starting concentration of 43.5 μg/ml) with an equal volume of antiserum (see Results for dilutions used). After 30 min (for immunofluorescence) or 20 h (for opsonization and quantitative radioactive binding assay), the residual titer or antibody binding was assayed and compared to that of the unabsorbed serum to which only buffer had been added. Absorption of antiserum with brain and liver homogenate (prepared as [8]) was performed by similar 30-min incubations of serial twofold dilutions of the tissue homogenate (initial concentration of 20 mg protein/ml) with equal volumes of antiserum. After centrifugation (3,000 *g*, 10 min) the residual antibody activity was assessed by immunofluorescence.

Indirect Radioactive Binding Assays. These were used to give a quantitative measure of antibody binding to thymocytes after inhibition of the rabbit IgG anti-rat brain Thy-1 antigen by Thy-1 antigen, and have been described (8).

Immunofluorescence. Indirect immunofluorescence was performed on frozen sections and on cell suspensions as described previously (30, 35).

Simultaneous immunofluorescence with rabbit and mouse antisera was performed as follows: cells in suspension were incubated with the first antiserum (20 min, 4°C, 10 mM NaN_3), washed, incubated with the second antiserum (20 min, 4°C, 10 mM NaN_3), washed and incubated sequentially with FITC-labeled goat anti-rabbit IgG (dilution 1:20) and FITC-labeled goat anti-mouse IgG (dilution 1:10). Routine controls included incubating the two antisera in reverse sequence, substituting normal rabbit and/or mouse serum for one or both antisera; and reacting the FITC and RITC conjugates against cells coated with antiserum from the inappropriate species (i.e. mouse instead of rabbit and vice versa).

Cocapping experiments using rabbit and mouse antisera were performed as follows. Cells in suspension were incubated with the first antiserum (20 min, 4°C) and developed with the appropriate conjugated antiserum under capping conditions (30 min, 37°C). The cells were washed in cold buffer containing 10 mM NaN_3, checked for capping, and smeared on glass slides in a cytocentrifuge (Shandon Southern Instruments, Inc., Sewickley, Pa.). The cells were then incubated with the second antiserum (20 min, 21°C), washed, and developed with the second conjugated antiserum. In a variation of this procedure, cells were incubated with the

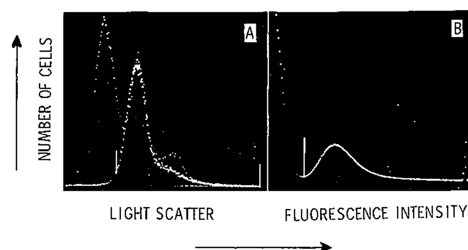

FIG. 1. Analysis of Thy-1-positive and Thy-1-negative populations of rat bone marrow cells by low angle (0°C) light scatter and fluorescence intensity using the FACS II, (Becton-Dickinson; laser power 0.4 W, wavelength 488 nm; photomultiplier voltage 650; 530 nm 3 cavity band pass filter, Ditric Optics; fluorescence gain 2; scatter gain 2). Adult DA strain rat bone marrow cells were reacted with FITC rabbit $F(ab^1)_2$ anti-rat brain Thy-1 antibodies as described in Materials and Methods and Table IV. (A) Scatter profile of fluorescing (Thy-1 positive) cells (large dots) superimposed on scatter profile of total cells (small dots). Approximately 80% of cells in center peak (lymphocytes) and 26% of cells in right peak (myeloid and blast cells) fluoresced. The threshold marker for excluding erythrocytes (left peak) during cell sorting was selected so as to remove fewer than 2% fluorescing (and nonfluorescing) nucleated cells. (B) Fluorescence profile of nucleated cells. The marker defines the threshold above and below which cells were sorted into fluorescing (Thy-1-positive) and nonfluorescing (Thy-1-negative) populations, respectively. The position of the marker coincides with the maximum fluorescence intensity displayed by bone marrow cells after incubation with anti-Thy-1 antibodies that had been absorbed with rat thymocytes. In this figure, 43.6% of nucleated cells were Thy-1 positive. A detailed description of the cell types represented in the Thy-1-positive and Thy-1-negative populations will be presented in a separate report.[2]

first antiserum under capping conditions (30 min, 37°C) and with the second antiserum under noncapping conditions (4°C, 10 mM NaN_3) before smearing and developing. Results with both procedures were identical. Routine controls were similar to those included in the simultaneous immunofluorescence experiments (see above). Additional control experiments are described in Results.

Separation of Thy-1-Positive and Thy-1-Negative Bone Marrow Cells. A Becton Dickinson fluorescence-activated cell sorter (FACS II) (36) was used to separate Thy-1-positive and Thy-1-negative cells from rat bone marrow. For these experiments 200×10^6 nucleated bone marrow cells from young adult DA, Lewis, and Wistar strain rats were incubated for 20 min at 4°C with 0.12–1.9 μg fluorescein-conjugated rabbit $F(ab')_2$ anti-rat brain Thy-1 antibodies in 0.25 ml Eisen's balanced salt solution. The cells were washed three times in buffer and diluted to a concentration of 5×10^6 cells/ml for sorting on the FACS. Erythrocytes were gated electronically according to their distribution profile under 0°C light scatter, using a threshold that excluded fewer than 2% of nucleated bone marrow cells (Fig. 1 A). The nucleated cells were sorted into Thy-1-positive and Thy-1-negative populations according to intensity of fluorescence as described under Results and Fig. 1 B.

Antibody Treatment of Donor Cells. Sensitization of donor cells with antibody was conducted as described previously (26). Briefly, 1×10^6–1×10^8 donor cells were incubated for 20 min at 4°C with 0.05 ml of appropriately diluted rabbit or mouse anti-Thy-1.1 serum or normal serum. The treated cells were washed twice in cold Tyrode's solution, checked for viability by trypan blue dye exclusion, and diluted for injection. The presence of mouse or rabbit immunoglobulin on the treated rat cell was verified by indirect immunofluorescence.

Spleen Weight and CFU-S Assays. 1×10^6–1×10^8 antibody-treated adult rat bone marrow, neonatal spleen, or fetal liver cells in 1.0 ml Tyrode's solution were injected i.v. into syngeneic rats that had received 800 rads whole body X-irradiation 2 h previously. 0.4×10^6–1×10^6 FACS separated adult rat bone marrow cells in 1.0–2.0 ml Eisen's balanced salt solution were injected i.v. into syngeneic rats that had received 750 rads whole body X-irradiation 6 h previously. The recipients were sacrificed 12 days after injection. The spleens were weighed, fixed, and examined for macroscopically visible colonies (26). Selected spleens were embedded in paraffin, sectioned, and stained with hematoxylin and eosin for microscopic examination of spleen colony morphology.

Complement-Mediated Cytotoxicity. 2.5×10^6 nucleated rat bone marrow, thymus, spleen, or lymph node cells in 25 μl Dulbecco's phosphate-buffered saline plus 10 mM NaN_3 were incubated (30 min, 4°C) with 50 μl appropriately diluted antiserum or normal serum. Cells were then washed once and incubated for 60 min at 37°C with 0.1 ml complement (normal Lewis rat serum). Viability counts were performed by trypan blue dye exclusion. The specificity and extent of the killing, as compared to the normal serum control, was confirmed by examination of the surviving cells by immunofluorescence (27).

Results

Distribution of Thy-1-Positive Cells in Rat Lymphohemopoietic Tissues. The distribution of putative Thy-1-positive cells in the lymphohemopoietic tissues of normal rats is shown in Table I. The highest percentages of positive cells are found in bone marrow, thymus and spleen; the lowest percentages are in lymph node, blood, and thoracic duct lymph. The same proportions of positive cells were obtained in indirect immunofluorescence assays and in complement-mediated cytotoxicity assays using two different lots of rabbit anti-rat brain Thy-1 serum (Materials and Methods).

In frozen sections of rat thymus the rabbit $F(ab')_2$ anti-Thy-1 antibodies (serum 2) labeled cortical thymocytes much more intensely that it did medullary thymocytes. Consistent with this was the finding that strongly Thy-1-positive thymocytes are sensitive to cortisone. The 53% reduction produced by this steroid in the proportion of Thy-1-positive thymocytes (Table I) represents an absolute decrease of 92% in the total number of Thy-1-positive cells. Thy-1-positive cells in bone marrow and spleen are similarly sensitive to cortisone (Table I).

Inhibition of the Binding of Rabbit IgG Anti-Rat Brain Thy-1 to Thymocytes. To assess the specificity of the reaction of the rabbit anti-rat brain Thy-1 antiserum, the binding of a constant amount (1.5 μg) of the IgG fraction of this antiserum (serum 1) to rat thymocytes was inhibited by a prior incubation of the antibody with serial twofold

TABLE I

Distribution of Thy-1-Positive Cells in Rat Lymphohemopoietic Tissues

Tissue‡	Per cent Thy-1-positive cells (mean ± SE)*	
	Untreated	Cortisone-treated§
Bone marrow	21.0 ± 1.7	5.2 ± 0.8
Thymus	92.0 ± 1.2	43.4 ± 2.1
Spleen	19.3 ± 2.0	4.1 ± 0.4
Lymph node	3.6 ± 0.5	5.5 ± 0.6
Blood	7.2 ± 1.9	ND‖
Thoracic duct lymph	8.1 ± 0.4	ND
Neonatal spleen (3 day)	36.6 ± 3.2	ND
Fetal liver (16 day)	1.2 ± 0.3	ND

* Determined by indirect immunofluorescence using rabbit $F(ab')_2$ anti-rat brain Thy-1 at a final concentration of 0.08 mg protein/ml; normal rabbit $F(ab')_2$ at the same concentration was used as a control. Values given are the net percent of nucleated cells that fluoresced. Note for fetal liver this includes erythrocytes. Mean of three to five experiments.

‡ Cells from (Lewis × DA)F_1 hybrid rats, 8- to 10-wk-old for the adult tissues.

§ Donors injected i.m. 3 days previously with 50 mg cortisone acetate per 100 g body weight.

‖ ND, not done.

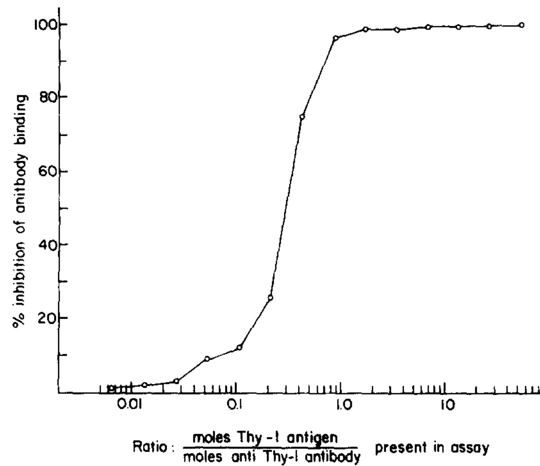

FIG. 2. Inhibition by pure Thy-1 antigen of rabbit IgG anti-rat brain Thy-1 serum. Duplicate 90-μl aliquots of the antigen, in doubling dilutions from 1.04 to 0.1 μg were incubated at 4°C for 20 h with 1.5 μg of the IgG fraction of the rabbit anti-rat brain Thy-1 serum (giving a final antibody dilution equivalent to 1:1,600 of the serum). Rat thymocytes (30 μl at 2×10^8/ml) were then added, and the antibody binding to these measured by the indirect radioactive binding assay (8). The binding of unabsorbed serum (38,000 cpm) and of serum absorbed with an excess (4×10^7) of rat thymocytes (2,450 cpm) was also measured. The specific binding of unabsorbed serum (35,550 cpm) was equivalent to 0.78 pmol of rabbit IgG bound (calculated as in references 8 and 47). The specific binding of the antiserum absorbed with Thy-1 antigen was then expressed as a percent of the maximum specific binding, and this was subtracted from 100% to give the percent inhibition obtained (ordinate). The moles of Thy-1 present at each point (mol wt 24,000 daltons) (10) were divided by the moles of antibody present (0.78 pmol) to give the molar ratio shown on the abscissa.

dilutions of pure Thy-1 antigen. More than 90% of the inhibition occurred in the range of 2–10 ng of Thy-1 antigen; and 25 ng reduced binding to the background level. It can be seen in Fig. 2 that the inhibition occurs steeply around the equimolar ratio of antigen to antibody.

Two probable sources of error in the calculation of the amount of Thy-1 antigen should be noted. This was estimated by the Lowry et al. method (34) for protein determination, using bovine serum albumin as the standard. The latter protein has 1.68 the tyrosine content of Thy-1 (37, 38) (the major amino acid measured in this protein determination); moreover bovine serum albumin is not a glycoprotein, whereas 29% by weight of rat brain Thy-1 is carbohydrate whose mass is obviously not assayed in the protein determination. Both these sources of error would lead to an underestimate of the true amount of Thy-1, by as much as 2.4-fold in all, so that the antigen: antibody ratio in Fig. 2 almost certainly underestimates the true ratio, possibly by as much as this factor.

Effect of Rabbit Anti-Rat Brain Thy-1 Antiserum on CFU-S in Bone Marrow. Results in Table II show that the rabbit anti-Thy-1 antiserum (serum 1) in dilutions from 1:100 to 1:5,000, was able to completely abrogate the spleen CFU-S activity of adoptively transferred adult rat bone marrow cells. 50% inhibition of CFU-S activity was observed at a 1:10,000 dilution of the antiserum. A similar degree of inhibition by the antiserum of the ability of bone marrow cells to increase spleen weight in irradiated recipients was observed. Prior absorption of the antiserum with pure rat brain Thy-1 antigen removed the ability of the antiserum to inhibit CFU-S activity and to prevent

TABLE II

Effect of Anti-Thy-1 Serum on CFU-S in Rat Bone Marrow

Treatment of bone marrow cells*			Parameters measured (mean ± SE)‡	
Antiserum (dilution)§		Absorbed with Thy-1 antigen ‖	Spleen colonies	Spleen weight
Normal rabbit serum				
	(1:100)	—	13.9 ± 0.8	127 ± 10
	(1:100)	2.00	14.6 ± 0.9	138 ± 13
Anti-Thy-1	(1:100)	—	1.6 ± 0.3	98 ± 9
	(1:500)	—	1.1 ± 0.3	95 ± 8
	(1:500)	2.00	13.8 ± 0.5	140 ± 11
	(1:500)	0.67	12.8 ± 1.0	130 ± 12
	(1:500)	0.33	12.4 ± 0.3	145 ± 10
	(1:2,500)	—	1.6 ± 0.4	88 ± 7
	(1:5,000)	—	0.7 ± 0.3	98 ± 16
	(1:10,000)	—	6.3 ± 0.9	154 ± 42
	(1:20,000)	—	13.0 ± 1.5	139 ± 24

* See Materials and Methods for details.

‡ 1×10^6 nucleated bone marrow cells from 8- to 10-wk-old (Lewis × DA)F_1 strain rats were injected i.v. into irradiated (800 rads) 4- to 5-wk-old syngeneic recipients. Each entry represents the mean of six experiments (total 12–24 surviving animals per entry). The data were collected 12 days after injection of donor cells. Values for irradiated rats not given bone marrow cells were: spleen colonies, 2.0 ± 0.5; spleen weight, 54 ± 7.

§ Rabbit anti-rat brain Thy-1 antiserum (serum 1).

‖ The amount of purified Thy-1.1 antigen shown, in 125 μl, was added to 125 μl of diluted serum 16 h before treatment of bone marrow cells.

spleen weight gain. Allowing for the 1.8-fold larger vol and 4.9-fold higher concentration of the antiserum used in this assay, 0.33 μg Thy-1 used here is equivalent to 37 ng used in Fig. 2, or an antigen: antibody molar ratio of 1.9.

Further evidence of the specificity of the reaction against stem cells was obtained using two murine anti-Thy-1.1 alloantisera. AKR/Cum anti-AKR/Jax thymocyte antiserum (dilution 1:20) was found to completely inhibit CFU-S activity in adult rat bone marrow. This inhibition was prevented by prior absorption of the antiserum with purified rat brain Thy-1 antigen, or with AKR/Jax (Thy-1.1 type) but not AKR/Cum (Thy-1.2 type) brain homogenates. CBA mouse anti-AKR mouse thymocyte serum (dilution 1:20) was similarly effective in inhibiting the CFU-S activity of adult rat bone marrow.

Effect of Anti-Thy-1 Antiserum on CFU-S in Neonatal Spleen and Fetal Liver. In Table IIIa the increase in spleen weight and number of spleen colonies after injection of increasing numbers of spleen cells from 3-day-old rats is shown. Incubation of the highest number of cells used (10^7) with increasing dilutions of rabbit anti-rat brain Thy-1 serum inhibited the CFU-S activity as assessed by both parameters (Table IIIb). An approximately fourfold higher concentration of antiserum than that required for the adult bone marrow cells was needed for comparable inhibition, reflecting the larger cell inoculum used and higher proportion of Thy-1-positive cells in the neonatal spleen.

The mean spleen weights obtained with increasing numbers of neonatal spleen cells, and with 10^7 spleen cells treated with various dilutions of anti-Thy-1 antiserum,

are plotted in Fig. 3. It can be seen that not only is the relationship of spleen weight to numbers of injected cells approximately linear, so also is the abrogation of this activity by anti-Thy-1-serum. Moreover, the dose-response curves are virtually coincidental.

The rabbit anti-rat brain serum was similarly effective in eliminating CFU-S activity in 16 day fetal rat liver.

CFU-S Activity in Thy-1-Positive and Thy-1-Negative Populations of Bone Marrow Cells Separated by the Fluorescence-Activated Cell Sorter (FACS). Bone marrow cells from young adult DA strain rats were incubated in vitro with fluorescein-conjugated rabbit $F(ab^1)_2$ anti-rat brain Thy-1 antibodies (serum 1). Thy-1-positive and Thy-1-negative populations of nucleated cells were separated on the FACS. Almost complete separation (>98% purity) of these populations was possible because of the sharp bimodality of fluorescence intensity (Fig. 1).

Between 38.5 and 49.0% (mean 44.9%) of nucleated bone marrow cells were Thy-1 positive by FACS analysis.[4] The percentage of Thy-1-positive cells was constant at antibody concentrations of 0.5–7.5 μg protein/ml/8 $\times$ 10^8 nucleated cells. Antibody activity was reduced to background levels by absorption with rat thymocytes.

Results in Table IV show that all CFU-S in DA rat bone marrow are Thy-1 positive. In a series of four experiments using FACS separated cells, 1 $\times$ 10^6 Thy-1-positive bone marrow cells produced a mean of 59.6 hemopoietic colonies in the spleens of irradiated recipients; whereas 1 $\times$ 10^6 antibody-treated but unseparated bone marrow cells, containing a mean of 40.1% Thy-1-positive cells, produced a mean of 25.3 spleen colonies. This represents a 2.6-fold enrichment in CFU-S activity (or 100% recovery) when the data are corrected for a mean of 3.3 endogenous colonies. In contrast, 1 $\times$ 10^6 FACS separated Thy-1-negative cells failed to produce spleen colonies above background levels.

Complete recovery of CFU-S activity in the Thy-1-positive cell fraction was also obtained with FACS separated Lewis strain (two experiments) and Wistar strain (one experiment) rat bone marrow cells.

In another series of experiments, Thy-1-positive cells were sorted on the FACS according to relative fluorescence intensity. More than 90% of CFU-S activity was recovered within the upper 25th percentile of fluorescent cells.

In none of the above experiments did the $F(ab^1)_2$ anti-Thy-1 antibodies, used at a concentration of 1.9 μg/ml/8 $\times$ 10^8 nucleated bone marrow cells, alter the ability of CFU-S to migrate to spleen and to form hemopoietic colonies there (compare antibody-treated to untreated bone marrow cell controls in Table IV). However, antibody concentration of 7.5 μg/ml/8 $\times$ 10^8 cells caused an approximately 50% decrease in the number of spleen colonies, due at least in part to agglutination of Thy-1-positive cells.

Relation of the Thy-1 Antigen to the Rat BMLA. In the rat (5, 6, and this paper) and the mouse (data not shown) the same proportions of thymus, spleen, lymph node, and bone marrow cells that react with anti-Thy-1 serum also react with antiserum to the

[4] Values for Thy-1-positive cells similar to those determined by FACS analysis were obtained by fluorescence microscopy. These values are significantly greater than those recorded in Table I (45 versus 21% Thy-1-positive bone marrow cells). In as much as the reagents and sensitivities of the immunofluorescence assay systems were identical, the disparity in results probably reflects differences between the two stocks of DA strain rats that were used (Materials and Methods). Such intrastrain differences in percentage of Thy-1-positive rat bone marrow cells have been reported by Hunt et al. (17).

TABLE III

*Abrogation of CFU-S Activity in Rat 3 Day Spleen by Rabbit Anti-Rat Brain Thy-1 Antiserum**

A. Spleen weight gain and colony formation induced by 3 day spleen cells: dose-response relationship

Cell dose‡	Spleen weight ± SE	Spleen colonies ± SE
	mg	
None	74.1 ± 4.5	2.1 ± 0.5
5×10^5	74.2 ± 8.9	7.4 ± 1.5
1×10^6	81.8 ± 9.9	13.4 ± 3.1
5×10^6	120.6 ± 15.1	38.0 ± 8.1
1×10^7	149.0 ± 19.6	TNTC§

B. The abrogation of CFU-S activity in 1×10^7 3 day rat spleen cells by treatment with rabbit anti-rat brain Thy-1 serum

Dilution of anti-Thy-1 serum	Spleen weight ± SE	Spleen colonies ± SE
	mg	
1:500	70.5 ± 5.2	3.6 ± 2.5
1:1,250	85.9 ± 16.2	4.1 ± 1.0
1:2,500	104.1 ± 10.8	6.6 ± 1.1
1:5,000	119.6 ± 8.8	34.9 ± 5.9
1:10,000	142.0 ± 32.8	TNTC
Normal rabbit serum		
1:500	169.8 ± 23.0	TNTC

* Data represents the mean values for survivors from three to four experiments, 3–12 animals per point.

‡ Cell numbers are total cells injected, including erythrocytes.

§ TNTC, too numerous to count.

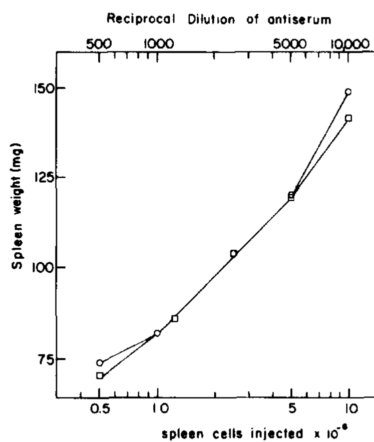

FIG. 3. Spleen weight response to increasing numbers of 3 day donor spleen cells and to increasing dilutions of rabbit anti-rat brain Thy-1 serum. (○) is the mean spleen weight in response to increasing cell dose (bottom abscissa); (□) is the mean spleen weight in response to 10^7 spleen cells treated with increasing dilutions of rabbit anti-rat brain Thy-1 serum. Data from Table III.

bone marrow lymphocyte antigen (anti-BMLA) (27). Both antisera have been shown to abrogate CFU-S activity in adult rat bone marrow (26, and this paper).

That the Thy-1 and BMLA antigenic determinants are present on the same

TABLE IV

CFU-S Activity in Thy-1-Positive and Thy-1-Negative Populations of DA Rat Bone Marrow Cells Separated by the FACS

Fraction	Treatment of bone marrow cells			Spleen colonies (mean ± SE) ‖
	Incubated with anti-Thy-1*	Separated by FACS‡	Per cent Thy-1 positive§	
Buffer control	—	—	—	3.3 ± 0.6
Untreated, unseparated	No	No	—	28.5 ± 2.3
Treated, unseparated	Yes	No	40.1	25.3 ± 2.2
Thy-1 positive	Yes	Yes	>98	59.6 ± 5.8
Thy-1 negative	Yes	Yes	<2	3.3 ± 0.7

* FITC rabbit $F(ab')_2$ anti-rat brain Thy-1 antibodies were used at a final concentration of 1.9 μg protein/ml/8 × 10^8 nucleated bone marrow cells.

‡ See Fig. 1 for conditions of cell separation.

§ Percent of nucleated cells.

‖ Irradiated (750 rads) syngeneic recipients were injected i.v. with buffer or with 0.4 × 10^6–1.0 × 10^6 nucleated bone marrow cells. Spleen colonies were counted on day 12. Data represents the mean values for survivors from four experiments, 12–16 animals per point, normalized to 1 × 10^6 injected cells.

TABLE V

Cocapping of BMLA and Thy-1.1 Antigens on Rat Thymocytes

Experimental protocol*		Immunofluorescence pattern‖
First antiserum + developing antiserum	Second antiserum + developing antiserum	
(Capping conditions)‡	(Noncapping conditions)§	
NRS + Fl-GAR-Ig	NMS + Rh-GAM-Ig	No staining; no capping
NRS + Fl-GAR-Ig	M-anti-Thy-1.1 + Rh-GAM-Ig	Rh staining; no capping
R-anti-BMLA + Fl-GAR-Ig	NMS + Rh-GAM-Ig	Fl capping only
R-anti-BMLA + Fl-GAR-Ig	M-anti-Thy-1.1 + Rh-GAM-Ig	Fl and Rh cocapping
NMS + Rh-GAM-Ig	NRS + Fl-GAR-Ig	No staining; no capping
NMS + Rh-GAM-Ig	R-anti-BMLA + Fl-GAR-Ig	Fl staining; no capping
M-anti-Thy-1.1 + Rh-GAM-Ig	NRS + Fl-GAR-Ig	Rh capping only
M-anti-Thy-1.1 + Rh-GAM-Ig	R-anti BMLA + Fl-GAR-Ig	Fl and Rh cocapping
R-anti-T + Fl-GAR-Ig	M-anti-Thy-1,1 + Rh-GAM-Ig	Rh staining; F1 capping
M-anti-Thy-1.1 + Rh-GAM-Ig	R-anti-T + Fl-GAR-Ig	Fl staining; Rh capping

* See Materials and Methods for details. NRS, normal rabbit serum; NMS, normal mouse serum; R-anti-BMLA, rabbit antiserum to rat bone marrow lymphocyte antigen; M-anti-Thy-1.1, AKR/cum mouse anti-AKR/J mouse thymocyte serum; R-anti-T, rabbit antiserum to rat T cells; Fl-GAR-Ig, FITC goat IgG anti-rabbit IgG; Rh-GAM-Ig, RITC goat IgG anti-mouse IgG.

‡ 37°C, 30 min.

§ 4°C, 10 mM NaN_3, 20 min.

‖ Fl, fluorescein (green) fluorescence; Rh, rhodamine (red) fluorescence; staining, uniform circumferential pattern of surface fluorescence; capping, broken polar pattern of surface fluorescence; cocapping, complete superimposition of Fl and Rh caps on cell surface.

molecule was demonstrated by double immunofluorescence in which the appropriate fluorescein- and rhodamine-conjugated antisera were used simultaneously to detect both antigens (Materials and Methods). All rat thymocytes and bone marrow cells which had Thy-1.1 antigen also had BMLA, and vice versa. Moreover, capping of either of these antigens with its appropriate antiserum caused the capping of the other antigen. Results of an experiment with thymocytes are presented in Table V; similar

results were obtained with bone marrow cells.

As a control for the specificity of the cocapping phenomenon, rabbit anti-T-cell serum (absorbed with rat bone marrow cells) was substituted for anti-BMLA in the above system. Although the anti-T-cell serum caused complete capping of the relevant T-cell antigen(s) on thymocytes, cocapping with Thy-1.1 antigen did not occur. Conversely, capping with anti-Thy-1.1 serum did not cause cocapping of antigen(s) detected by the anti-T-cell serum.

The conclusion that Thy-1 and BMLA are on the same molecule was further substantiated by the following observations. Binding of anti-BMLA serum to rat bone marrow, thymus, and spleen cells was completely prevented by absorption with small quantities of rat or AKR/J mouse brain homogenate. Anti-BMLA serum was neutralized by purified Thy-1 antigen used at the minimum concentration required to neutralize rabbit anti-Thy-1 serum of equal titer.

Discussion

We have used three experimental approaches to demonstrate that Thy-1 antigen is present on pluripotent stem cells in rat hemopoietic tissues: (*a*) depletion of Thy-1-positive cells by treatment with anti-Thy-1 antibodies; (*b*) isolation of Thy-1-positive and Thy-1-negative cells by the FACS; and (*c*) demonstration that Thy-1 and BMLA antigens are present on the same molecule. The CFU-S assay was used to specifically identify and quantify pluripotent stem cells. This assay detects clones of erythrocytic and myelocytic cells that are formed by individual stem cells; the number of colonies formed being a linear function of the number of hemopoietic cells injected. A derivative assay, the spleen weight assay, was used as a general measure of hemopoietic regenerative capacity.

Rabbit antiserum to purified rat brain Thy-1 molecule was used in the first set of experiments. The CFU-S activity of adult bone marrow was completely abrogated by dilutions of antiserum up to 1:5,000; and 50% inhibition was observed at 1:10,000. At such dilutions it seems unlikely that any antibodies except those to the Thy-1 antigen could be responsible. Moreover, this inhibition was completely neutralized by purified rat brain Thy-1 antigen, used here at antigen: antibody molar ratios of as little as 1.9:1. Other evidence of the specificity of the anti-Thy-1 serum was provided by antibody binding studies using thymocytes as target cells. Inhibition of antibody binding by purified rat brain Thy-1 antigen occurred steeply around the equimolar antigen: antibody range. Under these conditions, any minor contaminants of the purified antigen which might have escaped detection on the sodium dodecyl sulfate polyacrylamide gels could not have inhibited the action of the anti-Thy-1 serum.

The conclusion that the effect of the rabbit antiserum on rat CFU-S activity was specifically mediated by anti-Thy-1 antibodies was further substantiated by using two murine anti-Thy-1 alloantisera. The first of these, the AKR/Cum anti-AKR/J thymocyte serum, is raised between two mouse strains for which Thy-1 is the only known cell surface antigenic difference (39). That the abrogation of CFU-S activity was due to anti-Thy-1.1 antibodies in this serum was demonstrated by the neutralization of this effect by the rat brain Thy-1 antigen, and by absorption of the antiserum with the brain homogenate from the Thy-1.1 but not the Thy-1.2 type mouse strain. The second anti-serum, CBA anti-AKR thymocyte serum, which has been shown to only recognize the Thy-1.1 antigen on rat thymocytes by absorption with tissues from

Thy-1 congenic mouse strains (31), was equally effective in abrogating the CFU-S activity of adult rat bone marrow cells.

Additional evidence for the presence of Thy-1 antigen on hemopoietic stem cells is the finding that rabbit antiserum raised against a population of lymphocyte-like null cells from rat bone marrow (anti-rat bone marrow lymphocyte antigen serum; anti-BMLA serum) (27) reacts with Thy-1 antigen. Anti-BMLA serum has already been shown to eliminate CFU-S activity in adult rat bone marrow (26). Here we show that BMLA and Thy-1.1 occur on the same cells in rat bone marrow, and that antiserum to either antigen causes a redistribution (capping) of both antigens to the same area of the cell. Such cocapping has only been observed for antigens which are physically linked, such as β-2 microglobulin and HLA on human lymphocytes (40). Since the purified rat brain Thy-1 antigen fully inhibits the binding of anti-BMLA to rat lymphocytes, this physical linkage of the two antigens must be by virtue of their being determinants on the same Thy-1 molecule.

It is clear from the preceding experiments that anti-Thy-1 sera, raised by diverse methods, can completely abrogate CFU-S activity in suspensions of adult rat bone marrow cells; and that this effect can be prevented by neutralization with Thy-1 antigen. It is not clear, however, that the suppression of CFU-S activity is due to reaction of anti-Thy-1 antibodies directly with CFU-S. Alternatively, the effect may be at the level of Thy-1-positive cells which regulate the proliferation of CFU-S, as demonstrated in the mouse (22, 23). Assuming that anti-Thy-1 antibodies do react with CFU-S, it is possible that they do so nonspecifically by binding to Fc or complement receptors.

Results of experiments using the FACS show unequivocally that Thy-1 antigen is present on CFU-S. Thus, all CFU-S activity originally present in unseparated bone marrow cell suspensions was recovered in the Thy-1-positive cell population; whereas no CFU-S activity was present in the Thy-1-negative population. The use of $F(ab^1)_2$ fragments of anti-Thy-1 antibodies precluded nonspecific reactions with Fc or complement receptors. It could be argued of course that hemopoietic stem cells have only trace amounts of Thy-1 antigen on their surface, and that one is dealing with a threshold phenomenon in a highly sensitive assay system. This is not the case. Results of experiments in which Thy-1-positive bone marrow cells were separated according to relative fluorescence intensity (and hence relative amounts of Thy-1 antigen) indicate that CFU-S are among the most highly fluorescent subsets of cells. The results also indicate that if accessory cells exist in rat bone marrow, they too must be strongly positive for Thy-1 antigen.

Given that Thy-1 antigen is present on hemopoietic stem cells in adult rat bone marrow, it was of interest to determine if this was also true for stem cells in neonatal spleen and fetal liver. Stem cells in the latter tissues have been shown to differ significantly from stem cells in adult bone marrow with respect to physical characteristics and proliferative capacities (41). The relationship between these two types of stem cells is not clear, but there is evidence to suggest that the former can give rise to the latter. The present experiments show that embryonic-type stem cells, like adult-type stem cells, are Thy-1 positive. CFU-S activity in suspensions of 16 day fetal liver cells was completely abrogated by treatment with rabbit anti-rat brain Thy-1 antiserum. At this stage of development, the majority of Thy-1 positive cells have the morphology of large, basophilic blast cells (18).

Anti-Thy-1 serum also abrogated stem cell activity in suspensions of 3 day neonatal spleen cells as assayed by spleen colony formation and spleen weight gain. At less than saturating antibody dilutions (1:1,250 to 1:10,000) spleen weight gain after the injection of 1×10^7 treated cells was directly proportional to the dilution of anti-Thy-1 serum used. The slope of the resultant curve (Fig. 3) was identical to that formed by the injection of 5×10^5–1×10^7 untreated cells. The correspondence of these two dose-response curves strongly suggests that the anti-Thy-1 antibodies acted directly on the stem cells.

The proportions of rat lymphohemopoietic cells that were labeled by anti-Thy-1 sera in the present study are in good agreement with reported values (5, 6), in particular the high percentage of Thy-1-positive cells in bone marrow (between 21 and 45% of nucleated cells), and the low percentages in lymph node and thoracic duct lymph (less than 10%) have been confirmed. In addition, most Thy-1-positive cells in rat bone marrow, spleen and thymus cortex were found to be cortisone sensitive. Assuming a frequency of 0.7 for pluripotent stem cells (42) and of 20% for Thy-1-positive cells, it is evident that the stem cells comprise a maximum of 3.5% of Thy-1-positive cells in rat bone marrow. Moreover, they appear to belong to a cortisone resistant subset of Thy-1-positive cells.[2]

The identity of the remainder of the Thy-1-positive bone marrow cells is only partially known. Using an in vitro colony-forming unit assay (43), we have shown that progenitors of myeloid cells and of some B cells are Thy-1 positive.[2] Ontogenetic studies of Ig-positive cells also support the notion that immature members of the B-cell series are Thy-1 positive (18). Moreover, Thy-1-positive bone marrow cells, isolated by the FACS, have been shown to generate B lymphocytes in irradiated recipients (17). However, it is not possible to tell whether this was due to the transfer of pluripotent stem cells and/or B cell progenitors.

It also seems likely that Thy-1 antigen is present on thymocyte progenitors in the rat, but an independent marker for such cells has yet to be identified in this species. One promising molecule in this respect is the enzyme terminal deoxynucleotidyl transferase (TdT), which in the rat is normally restricted to cortical thymocytes and to a small subset (1–4%) of cells in the bone marrow and prepubertal spleen (44–46).[5] Several lines of evidence suggest that at least some of these could be thymocyte progenitors (46–48).[6] Recently we have isolated highly enriched (greater than 90%) populations of viable TdT-positive cells from rat bone marrow.[7] Experiments are in progress to determine the developmental potential of such cells, all of which have the Thy-1 phenotype.

Summary

Three approaches were used to demonstrate the presence of Thy-1 antigen on the surface of pluripotent hemopoietic stem cells in the rat. In the first, stem cells from

[5] K. E. Gregoire et al. Ontogeny of terminal deoxynucleotidyl transferase positive cells in the rat. Manuscript in preparation.

[6] I. Goldschneider, et al. Induction of terminal deoxynucleotidyl transferase and Ly antigens in mouse bone marrow and spleen cells with thymosin: demonstration by immunofluorescence. Manuscript submitted for publication.

[7] I. Goldschneider, et al. Isolation of terminal deoxynucleotidyl transferase positive rat bone marrow cells on the fluorescence-activated cell sorter. Manuscript submitted for publication.

fetal liver, neonatal spleen, and adult bone marrow were prevented from forming hemopoietic colonies in the spleens of irradiated recipients spleen (colony-forming unit assay) by incubation with antibodies to Thy-1 antigen. Highly specific rabbit heteroantiserum to purified rat brain Thy-1 antigen and mouse alloantisera to Thy-1.1-positive thymocytes were equally effective. This inhibition was neutralized by purified Thy-1 antigen.

In a second series of experiments, Thy-1-positive and Thy-1-negative populations of nucleated bone marrow cells were separated by the FACS. All of the hemopoietic stem cell activity was recovered in the Thy-1-positive population. The stem cells were among the most strongly positive for Thy-1 antigen, being in the upper 25th percentile for relative fluorescence intensity.

The relationships of Thy-1 antigen to the rat bone marrow lymphocyte antigen (BMLA) was shown in a third series of experiments. Rabbit anti-BMLA serum, which is raised against a null population of lymphocyte-like bone marrow cells, has been shown to have anti-stem cell activity. Here we demonstrate by double immunofluorescence, cocapping, and differential absorption studies that Thy-1 and BMLA are parts of the same molecule.

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References

1. Reif, A. E., and J. M. V. Allen. 1964. The AKR thymic antigen and its distribution in leukemias and nervous tissue. *J. Exp. Med.* **120:**413.
2. Douglas, T. C. 1972. Occurrence of a theta-like antigen in rats. *J. Exp. Med.* **135:**1054.
3. Stern, P. L. 1973. Alloantigen on mouse and rat fibroblasts. *Nat. New Biol.* **256:**76.
4. Scheid, M. P., E. A. Boyse, E. A. Carswell, and L. J. Old. 1972. Serologically demonstrable alloantigens of mouse epidermal cells. *J. Exp. Med.* **135:**938.
5. Acton, R. T., R. J. Morris, and A. F. Williams. 1974. Estimation of the amount and tissue distribution of rat Thy-1.1 antigen. *Eur. J. Immunol.* **4:**598.
6. Williams, A. F. 1976. Many cells in rat bone marrow have cell-surface Thy-1 antigen. *Eur. J. Immunol.* **6:**526.
7. Hilgers, J., J. Haverman, R. Nusse, W. J. van Blitterswijk, F. J. Cleton, Ph.C. Hageman, R. van Nie, and J. Calafat. 1975. Immunologic, virologic and Genetic aspects of mammary tumor virus-induced cell surface antigens: presence of these antigens and the Thy-1.2 antigen on murine mammary gland and tumor cells. *J. Natl. Cancer Inst.* **54:**1323.
8. Morris, R. J., and A. F. Williams. 1975. Antigens on mouse and rat lymphocytes recognized by rabbit antiserum against rat brain: the quantitative analysis of a xenogeneic antiserum. *Eur. J. Immunol.* **5:**274.
9. Morris, R. J., M. Letarte-Muirhead, and A. F. Williams. 1975. Analysis in deoxycholate of three antigenic specificities associated with the rat Thy-1 molecule. *Eur. J. Immunol.* **5:**282.
10. Barclay, A. N., M. Letarte-Muirhead, and A. F. Williams. 1975. Purification of the Thy-1 molecule from rat brain. *Biochem. J.* **151:**699.
11. Letarte-Muirhead, M., A. N. Barclay, and A. F. Williams. 1975. Purification of the Thy-1 molecule, a major cell-surface glycoprotein of rat thymocytes. *Biochem. J.* **151:**685.
12. Trowbridge, I. S., and R. Hyman. 1975. Thy-1 variants of mouse lymphomas: biochemical

characterization of the genetic defect. *Cell.* **6**:279.

13. Arndt, R., R. Stark, P. Klein, A. Muller, and H-G. Thiele. 1976. Solubilization and molecular characterization of membrane-bound antigens shared by thymocytes and brain. *Eur. J. Immunol.* **6**:333.
14. Zweiner, R. K., P. A. Barstad, and R. T. Acton. 1977. Isolation and characterization of murine cell surface components. I. Purification of milligram quantities of Thy-1.1. *J. Exp. Med.* **146**:986.
15. Raff, M. C. 1971. Surface antigenic markers for distinguishing T and B lymphocytes in mice. *Transplant. Rev.* **6**:52.
16. El-Arini, M. O., and D. Osoba. 1973. Differentiation of thymus-derived cells from precursors in mouse bone marrow. *J. Exp. Med.* **137**:821.
17. Hunt, S. V., D. W. Mason, and A. F. Williams. 1977. In rat bone marrow Thy-1 antigen is present on cells with membrane immunoglobulin and on precursors of peripheral B lymphocytes. *Eur. J. Immunol.* **7**:817.
18. Ritter, M. A., L. K. Gordon, and I. Goldschneider. Distribution and identity of Thy-1 bearing cells during ontogeny in rat hemopoietic and lymphoid tissues. *J. Immunol.* In press.
19. Tynan, M. L. 1975. Allogeneic radiation chimeras: long term studies. *Transplantation. (Baltimore).* **19**:326.
20. von Boehmer, H., L. Hudson, and J. Sprent. 1975. Collaboration of histocompatible T and B lymphocytes using chimeras from tetraparental bone marrow chimeras. *J. Exp. Med.* **142**:989.
21. Thierfelder, S. 1977. Haemopoietic stem cells of rats but not of mice express Thy-1.1 alloantigen. *Nature (Lond.).* **269**:691.
22. Wiktor-Jedrzejczak, W., S. Sharkis, A. Ahmed, K. Sell, and G. W. Santos. 1977. "Theta-sensitive cell and erythropoiesis: identification of a defect in W/WV anemic mice". *Science (Wash. D. C.).* **196**:313.
23. Shinpock, S. C., and J. W. Goodman. 1977. Effect of thymocytes on CFU-S kinetics. *Exp. Hemat.* **5** (Suppl. 2):36.
24. Till, J. E., and E. A. McCulloch. 1961. A direct measurement of the sensitivity of normal mouse bone marrow cells. *Radiat. Res.* **14**:213.
25. Thomas, D. B. 1973. The radiation chimera as an experimental model for the study of haemopoietic stem cell populations. *In* Haemopoietic Stem Cells, Ciba Foundation Symposium. Elsevier Scientific Publishing Co., Amsterdam, Netherlands **13**:71–98.
26. Goldschneider, I. 1977. Heteralogous antiserum to rat lymphohemopoietic precursor cells. *J. Immunol.* **118**:2040.
27. Goldschneider, I. 1976. Antigenic relationships between bone marrow lymphocytes, cortical thymocytes, and a subpopulation of peripheral T cells in the rat: description of a bone marrow lymphocyte antigen. *Cell. Immunol.* **24**:289.
28. Wilson, D. B. 1967. Quantitative studies on mixed lymphocyte interactions in rats. I. Conditions and parameters of response. *J. Exp. Med.* **126**:625.
29. Boyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. Clin. Lab. Invest.* **21** (Suppl. 97):1.
30. Goldschneider, I., and D. D. McGregor. 1973. Anatomical distribution of T and B lymphocytes in the rat: development of lymphocyte-specific antisera. *J. Exp. Med.* **138**:1443.
31. Williams, A. F., A. N. Barclay, M. Letarte-Muirhead, and R. J. Morris. 1976. The tissue distribution, purification and chemical composition of the rat Thy-1 antigens. *Cold Spring Harbor Symp. Quant. Biol.* **46**:51.
32. Nisonoff, A., F. C. Wissler, L. N. Lipmann, and D. L. Woernley. 1960. Separation of univalent fragments from the vibalent antibody molecule by reduction of disulfide bonds. *Arch. Biochem. Biophys.* **89**:230.
33. The, T. H., and E. W. Feltkamp. 1970. Conjugation of fluorescein isothiocyanate to antibodies. I. Experiments on the conditions of conjugation. *Immunology.* **18**:865.

34. Lowry, D. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265.

35. Goldschneider, I. 1975. Antigenic relationship between medullary thymocytes and a subpopulation of peripheral T cells in the rat: description of a masked antigen. *Cell. Immunol.* **16**:269.

36. Loken, M. R., and L. A. Herzenberg. 1975. Analysis of cell populations with a fluorescence-activated cell sorter. *Ann. N.Y. Acad. Sci.* **254**:163.

37. Barclay, A. N., M. Letarte-Muirhead, A. F. Williams, and R. A. Faulkes. 1976. Chemical characterization of the Thy-1 glycoproteins from the membrane of rat thymocytes and brain. *Nature (Lond.).* **263**:563.

38. Peters, T., and C. Hawn. 1967. Isolation of two large peptide fragments from the amino and carboxyl-terminal positions of bovine serum albumin. *J. Biol. Chem.* **242**:1566.

39. Acton, R. I., E. P. Blankenhorn, T. C. Douglas, R. D. Owen, J. Hilgers, H. A. Hoffman, and E. A. Boyse. 1973. Variations among sublines of inbred AKR mice. *Nat. New Biol.* **245**:8.

40. Poulik, M. D., M. Bernoco, D. Bernoco, and R. Ceppellini. 1973. Aggregation of HL-A antigens at the lymphocyte surface induced by antiserum to β_2-microglobulin. *Science (Wash. D. C.).* **182**:1352.

41. Metcalf, D., and M. A. S. Moore. 1971. Haemopoietic Cells. North-Holland Publishing Company, Amsterdam, Netherlands.

42. van Bekkum, D. W. 1977. The appearance of the multipotential hemopoietic stem cell. *In* Experimental Hematology Today. S. J. Baum and G. D. Ledney, editors. Springer, New York. 3.

43. Metcalf, D. 1977. Hemopoietic colonies. Recent Results in Cancer Research. Springer-Verlag, New York. Volume 61.

44. Barton, R. W., I. Goldschneider, and F. J. Bollum. 1976. The distribution of terminal deoxynucleotidyl transferase (TdT) among subsets of thymocytes in the rat. *J. Immunol.* **116**:462.

45. Goldschneider, I., K. E. Gregoire, R. W. Barton, and F. J. Bollum. 1977. Demonstration of terminal deoxynucleotidyl transferase in thymocytes by immunofluorescence. *Proc. Natl. Acad. Sci. U.S.A.* **74**:734.

46. Gregoire, K. E., I. Goldschneider, R. W. Barton, and F. J. Bollum. 1977. Intracellular distribution of terminal deoxynucleotidyl transferase (TdT) in bone marrow and thymus. *Proc. Natl. Acad. Sci. U.S.A.* **74**:3993.

47. Silverstone, A., H. Cantor, G. Goldstein, and D. Baltimore. 1976. Terminal deoxynucleotidyl transferase is found in prothymocytes. *J. Exp. Med.* **144**:543.

48. Pazmino, N. H., J. N. Ihle, and A. L. Goldstein. 1978. Induction *in vivo* and *in vitro* of terminal deoxynucleotidyl transferase by thymosin in bone marrow cells from athymic mice. *J. Exp. Med.* **147**:708.