DIFFERENTIATION OF THYMUS-DERIVED CELLS FROM PRECURSORS IN MOUSE BONE MARROW*

BY M. O. EL-ARINI AND D. OSOBA‡

(From The Institute of Medical Science and Department of Medicine, University of Toronto, and the Ontario Cancer Institute, Toronto, Ontario, Canada)

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Although the thymus-derived (T) cells and their progeny (effector cells) that are involved in allograft immunity and in humoral immunity have been extensively studied, very little is known about the differentiation of T cells from their progenitors. It is assumed that mouse bone marrow contains pluripotent stem cells, since transplantation of marrow into heavily irradiated recipients results in repopulation of the myeloid and lymphoid systems (1-5). The repopulation of at least a portion of the lymphoid system could conceivably come from T cells which might be present in the marrow. Alternatively, repopulation may be achieved from more primitive precursors. If these two types of cells have different physical properties, then it should be possible to distinguish between these alternatives by using equilibrium density centrifugation as a means of separating cells in mouse bone marrow.

Materials and Methods

Mice.—Male C57BL/6J, DBA/2J, and C3H/HeJ mice, obtained from Jackson Laboratory, Bar Harbor, Maine, were used in the experiments when they were 7-9 wk old.

Cell Suspensions.—Suspensions of spleen cells in phosphate-buffered saline (PBS) were prepared as described previously (6). Bone marrow cell suspensions were prepared by removing both femora and flushing the cells out of the marrow cavity with PBS. The resulting clumps of cells were passed through a fine needle to produce a single-cell suspension. Cells designated for the density separation procedure were washed and suspended in the buffer to be used in the density gradient. For the mixed leukocyte reaction (MLR), the cells were suspended in tissue culture medium as previously described (6). For transplantation, the cells were suspended in PBS.

Cell Separation.—C57BL/6J marrow cells were separated by density centrifugation on a linear gradient of Ficoll using the method described by Gorczynski et al. (7). By the addition of an appropriate amount of buffer, the Ficoll (Pharmacia, Uppsala, Sweden) was adjusted to

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‡ Associate of the Medical Research Council of Canada and Associate Professor, Department of Medicine, University of Toronto.

Abbreviations used in this paper: B cell, bone marrow-derived cell; CFU-S, hematopoietic colony-forming cells; FCS, fetal calf serum; GVHD, graft-vs.-host disease; MLR, mixed lymphocyte reaction; PB cells, progenitors of B cells; PBS, phosphate-buffered saline; PT cells, progenitors of T cells; T cell, thymus-derived cell.
294 mosmol with respect to the water content. The buffer pH was 5.5. From 2 to $4 \times 10^8$ marrow cells were uniformly distributed throughout both chambers of low- and high-density Ficoll. Then a linear gradient was generated by pumping low-density Ficoll into the chamber containing high-density Ficoll at half the rate of the outflow of the dense Ficoll. The gradient was prepared in a 30 ml cellulose nitrate tube (Beckman Instruments, Inc., Fullerton, Calif.) in a period of 30 min. Then the cells were centrifuged at 3,800 g for 45 min using an SW-25 rotor.

After centrifugation, 2-ml fractions were collected by upward displacement of the gradient using dense Ficoll as the displacing agent. Each fraction was mixed, and the refractive index read in a refractometer (Abby Refractometer, Bausch and Lomb, Inc., Rochester, N. Y.). The cells were washed once in PBS. A sample was removed, and after addition of Zaps-Isoton (Coulter Diagnostics, Inc., Hialeah, Fla.) the number of nucleated cells per fraction was counted with an electronic cell counter (Coulter Counter Model F, Coulter Electronics, Inc., Hialeah, Fla.). With the aid of a computer program the nucleated cell counts and the density per fraction were calculated. For culture, the cells in each fraction were adjusted to the desired concentration in an appropriate volume of tissue culture medium. The cells were kept at 4°C throughout these procedures.

**Mixed Leukocyte Cultures.**—The procedure was as described for mouse spleen cells (6). Briefly, the culture medium consisted of alpha medium minus nucleosides (Flow Laboratories, Inc., Rockville, Md.) supplemented with 10% fetal calf serum (FCS) (Flow Laboratories). Test cultures consisted of mixtures of "responding" marrow cells and allogeneic "stimulating" spleen cells. Control cultures consisted of responding cells equal to those used in the test cultures mixed with an appropriate number of heavily irradiated isogeneic spleen cells. In addition, a mixture of heavily irradiated responding cells and heavily irradiated stimulating cells was also incubated as a check on the effectiveness of the dose of irradiation in abolishing the response.

Test and control cultures were set up in triplicate for each experimental group. After a 16 h pulse with $[^3H]thymidine$, the cells were harvested at 136 h, since preliminary experiments showed the peak response to occur at this time.

**Spleen Colony Assay.**—We used the procedure described by Till and McCulloch (8). In summary, $5 \times 10^4$ bone marrow cells were injected intravenously into irradiated (800 rad) recipients; 9 days later the spleens were removed, fixed in Bouin's solution, and scored for macroscopic colonies.

**Irradiation Procedure.**—A 137Cs unit (9) was used to irradiate cells and mice. Stimulating cells in the MLR received 2,500 rad. In experiments involving transplantation of marrow, isogeneic recipients received 800 rad, while allogeneic recipients used for graft-vs.-host disease (GVHD) experiments received 1,200 rad 1-2 h before transplantation.

**51Cr Release Assay.**—We used the method described by MacDonald (10). Briefly, C57BL/6J bone marrow cells were mixed with irradiated (1,200 rad) DBA/2J spleen cells in plastic tubes (Falcon Plastics, Inc., Oxnard, Calif.) in a total volume of 3 ml of alpha medium plus 10% FCS. Triplicate cultures were used. After incubation for 6 days at 37°C in a humidified atmosphere of 5% CO$_2$ in air, sensitized cultures were resuspended with a Pasteur pipette. Serial dilutions of pooled cultures were incubated with $1 \times 10^7$ 51Cr-labeled DBA mastocytoma tumor cells in tissue culture medium for another 5-6 h. Then the assay cultures were diluted to a constant volume and spun at 1,000 rpm for 10 min. A constant proportion of supernatant was removed and counted in a well-type scintillation counter. Cultures containing no cells or unsensitized cells served as controls. Specific cytotoxicity was calculated as follows:

$$\text{Specific cytotoxicity} = \frac{\text{cpm (sensitized)} - \text{cpm (control)}}{\text{cpm (total)} - \text{cpm (control)}} \times 100.$$  

The specific cytotoxicity was plotted against the logarithm of the ratio of effector cells to target cells. Such a plot gives a series of parallel lines for cultures with different cytotoxic
activities (10). The relative number of effector cells in different cultures can be estimated by comparing, for the various groups, the effector to target cell ratio giving the same degree of cytotoxicity.

Anti-Ø Antibody.—Anti-Ø ascitic fluid was a generous gift from R. Gorczynski and was used as previously described (11). In summary, cells to be treated were suspended at 10⁶ cells/ml in standard medium. Anti-Ø ascitic fluid was added in a concentration of 1:5 or 1:2 and the mixture was incubated for 60 min at 37°C. At this time, the cells were washed and resuspended in a 10% solution of guinea pig serum in standard medium for another 45 min at 37°C. After this incubation the cells were washed three times in standard medium. Control experiments include treatment of cells with anti-Ø alone, complement alone, or no treatment.

RESULTS

Response of Mouse Bone Marrow Cells in the MLR.—Initially, we tested the capacity of unfractionated C57BL/6J mouse bone marrow to respond in the MLR. In several experiments, there was no response above background levels. Nevertheless, we reasoned that if mouse marrow contains MLR-responsive cells, the presence of such cells may be obscured by other cells capable of incorporating [³H]thymidine. However, if the density profile of these cells is different than that of MLR-responsive cells, the latter may become evident after separation of mouse bone marrow by density centrifugation. Therefore, C57BL/6J bone marrow cells were subjected to density centrifugation and from each fraction a sample of 10⁶ cells was mixed with 4 × 10⁶ heavily irradiated DBA/2J spleen cells. In some experiments, the light-density regions yielded an insufficient number of cells to enable an independent assay of each fraction. To overcome this problem, adjacent fractions were pooled to obtain the required number of cells for both the test and control cultures. Thus, the total number of cells per culture was constant for all regions of the gradient. Control cultures consisted of 10⁶ fractionated C57BL/6J cells mixed with 4 × 10⁶ heavily irradiated isogenic spleen cells. The results of a typical experiment are shown in Fig. 1 and in Table I. The total activity per fraction (Fig. 2) was obtained as follows:

\[
\text{Net response}/10^6 \times \frac{\text{Number of nucleated cells/fraction}}{10^6}
\]

The results show that after bone marrow cells are subjected to density centrifugation a profile of cells capable of responding in the MLR becomes apparent. The results of four experiments show that these cells have a modal density of 1.069 g/cm³ (SD ± 0.001).

Behavior of Mouse Bone Marrow Cells in the ⁵¹Cr Release Assay.—We studied the capacity of fractionated C57BL/6J bone marrow cells, sensitized in vitro against DBA/2J antigens, to lyse ⁵¹Cr-labeled DBA/2J mastocytoma cells. After density centrifugation, adjacent fractions containing cells responsive in the MLR (in the density region 1.06-1.08 g/cm³) were combined to give one pool (pool B) and adjacent fractions containing cells in the light-density region (1.05-1.059 g/cm³) were combined to give another pool of cells (pool A). Sam-
FIG. 1. The response by fractionated C57BL/6J bone marrow cells in the MLR; O—O, test cultures; X—X, control cultures. Each point represents the mean counts per minute in triplicate cultures; the bars represent 1 SE above and below the mean.

FIG. 2. Density profiles of C57BL/6J bone marrow cells capable of responding in the MLR (O—O) and capable of forming colonies (CFU-S) in the spleen (X—X).

Sple samples of 6 x 10⁶ cells from each pool and from unfractionated marrow were cultured with 8 x 10⁶ heavily irradiated DBA/2J spleen cells for 6 days. As a control, we cultured 6 x 10⁶ C57BL/6J spleen cells and 8 x 10⁶ irradiated DBA/2J spleen cells for a similar period of time. To test for immunological specificity in the response, we cultured C57BL/6J cells from each of these groups with irradiated spleen cells carrying different antigens than the sensitizing antigens,
i.e., C3H/HeJ spleen cells. After harvesting the cells in each group, they were tested for their capacity to lyse DBA/2J mastocytoma cells.

The results (Table I) show that after incubation with DBA/2J spleen cells for 6 days, unfractionated marrow contains very few effector cells (1% lysis). However, fractionated cells from pool B are enriched for effector cells (14% lysis), whereas cells from pool A did not produce any detectable lysis. Neither C57BL/6J spleen nor bone marrow cells sensitized to C3H/HeJ antigens produced lysis of DBA/2J target cells. Therefore, fractions of C57BL/6J bone marrow containing cells capable of responding to DBA/2J cells in the MLR also contain cells capable of becoming effector cells. However, the frequency of these cells is less than that present in suspensions of C57BL/6J spleen cells as determined from experiments done in parallel with the bone marrow experiments (Table I).

**TABLE I**

*Responses by Fractionated Bone Marrow Cells in Various Assays*

| Cells                      | Density region | MLR | CFU-S† | 51Cr release§ |
|----------------------------|----------------|-----|--------|---------------|
|                             | g/cm³          | cpm ± SE | colonies/spleen ± SE | %       |
| Fractionated bone marrow    | 1.05-1.059     | Test 3,750 ± 485 | 32 ± 0.7 | 0 |
|                            |                | Control 4,890 ± 720 |                      |        |
|                            |                | Net N.S.||        |
|                            | 1.06-1.08      | Test 11,225 ± 1,980 | 7 ± 2.5 | 14 |
|                            |                | Control 5,000 ± 690 |                      |        |
|                            |                | Net 5,625 ± 2,100 |                      |        |
| Bone marrow                | Unfractionated | Test 6,440 ± 2,590 | 10 ± 0 | 1 |
|                            |                | Control 5,190 ± 1,300 |                      |        |
|                            |                | Net 250 ± 2,890 |                      |        |
| Spleen                     | Unfractionated | Test 5,300 ± 1,730 | N.D.§ | 57 |
|                            |                | Control 270 ± 40 |                      |        |
|                            |                | Net 5,030 ± 1,730 |                      |        |

* 10⁶ C57BL/6J bone marrow cells were incubated with 4 × 10⁶ irradiated DBA/2J spleen cells for 5 days. The mean counts per minute in control cultures (10⁶ C57BL/6J bone marrow cells and 4 × 10⁶ irradiated C57BL/6J spleen cells) was subtracted from the mean counts per minute in the test cultures to obtain the net counts per minute per culture.

† Each irradiated recipient in the spleen colony assay received 5 × 10⁴ C57BL/6J bone marrow cells.

§ 6 × 10⁶ C57BL/6J bone marrow cells were incubated with 8 × 10⁶ irradiated DBA/2J spleen cells for 6 days. Then cells from these cultures were mixed with 10⁵ ⁵¹Cr-labeled DBA/2J mastocytoma cells and incubated for a further 5 h. As a control, 6 × 10⁶ C57BL/6J spleen cells were treated in a similar way.

|| Not significant.

¶ Not done.
Dose-Response Curve by Bone Marrow Cells Capable of Responding in the MLR.—We determined the dose-response curve by fractionated C57BL/6J bone marrow cells. Adjacent fractions containing cells capable of responding in the MLR were pooled; varying numbers of these cells (0.5–2 × 10⁶) were cultured with 4 × 10⁶ heavily irradiated DBA/2J spleen cells. Controls consisted of a similar number of responding cells cultured with isogeneic irradiated spleen cells. It is evident that within the range of cells cultured a linear dose-response relationship exists (Fig. 3). Furthermore, the intercept of the line obtained by the least-squares method did not differ significantly from zero (within 95% confidence limits).

Effect of Anti-θ Antibody on the MLR by Bone Marrow Cells.—MLR-responsive cells from fractionated C57BL/6J bone marrow cells were treated with anti-θ ascitic fluid and guinea pig serum (a source of complement). Controls consisted of fractionated untreated cells or cells treated with anti-θ ascitic fluid alone, or complement alone. We found that fractionated C57BL/6J bone marrow cells treated with anti-θ ascitic fluid and complement produced a much lower response in the MLR than did the cells in the control cultures (Table II).
Therefore, we conclude that mouse bone marrow contains T cells capable of responding in the MLR.

Density Profile of Hematopoietic Colony-Forming Cells (CFU-S).—Previous work has shown that CFU-S capable of forming colonies in the spleens of recipient mice are present in a light-density region of density gradients (12, 13). In order to have a base line for further experiments, we also determined the profile of CFU-S. C57BL/6J bone marrow cells were separated by density centrifugation, and samples of \(5 \times 10^4\) cells from each fraction were injected into irradiated isogeneic recipients. The total number of CFU-S per fraction was obtained as follows:

\[
\text{Number of colonies/spleen} \times \frac{\text{Number of nucleated cells/fraction}}{5 \times 10^4}.
\]

The profile of CFU-S was found to have a modal density of 1.06 g/cm\(^3\) (SD ± 0.002) (Fig. 2). This modal density is similar to that found for CFU-S in C57BL/6J spleen cells. Therefore, CFU-S in mouse bone marrow are found in a lighter density region of the gradient than are the cells capable of responding in the MLR.

Production of GVHD by Fractionated Bone Marrow Cells.—To determine the region of the gradient containing cells capable of producing acute GVHD, we followed the experimental protocol outlined in Fig. 4. C57BL/6J bone marrow cells were subjected to density centrifugation. Adjacent regions of the gradient enriched primarily for CFU-S (density region 1.05-1.059 g/cm\(^3\)) were combined to give one pool of cells (pool A). The remaining fractions enriched for cells responsive in the MLR (density region 1.06-1.08 g/cm\(^3\)) were combined to give a second pool of cells (pool B). Samples of \(4 \times 10^6\) cells from each pool were transplanted into heavily irradiated C3H/HeJ recipients. A third group (C) received \(4 \times 10^6\) C57BL/6J unseparated bone marrow cells. In addition, a

\[\text{El-Arini, M. O., and D. Osoba. Physical properties of mouse spleen cells involved in cell-mediated immunity. J. Immunol. In press.}\]
fourth control group of C3H/HeJ irradiated recipients was given $4 \times 10^6$ isogenic unseparated bone marrow cells. From previous studies (14) we reasoned that pools A and B contain sufficient CFU-S to prevent death from hematopoietic failure after irradiation. Therefore, isogenic bone marrow cells were not given to C3H/HeJ recipients receiving cells from each of these pools.

Mice receiving either unseparated allogeneic bone marrow cells (group C) or allogeneic cells from pool B of the gradient began to die of acute GVHD 12-14 days after transplantation (Fig. 5). All of the mice receiving cells from pool B were dead by day 20, while all of the mice receiving unfractionated allogeneic cells died by day 28. Mice receiving cells from pool A began to die from GVHD on day 21. However, 44% of them were still alive on day 45. Thus, mice receiving allogeneic cells from a region of the gradient enriched for MLR-
responsive cells died of GVHD at an earlier time than did mice receiving cells from regions of the gradient depleted for MLR-responsive cells, but enriched for CFU-S.

Repopulation of the Lymphoid System by Fractionated Bone Marrow Cells.— In these experiments, we attempted to determine which region of the gradient contains cells capable of repopulating the immunological system, as assayed by the capacity to give rise to cells which would respond in the MLR and produce acute GVHD. The experimental protocol is shown in Fig. 6. Based upon the density distribution of MLR-responsive cells and CFU-S in previous experiments, we divided the gradient into three regions and combined adjacent frac-

Day 0

CS7BL/6J
Bone marrow

Fractionate

Pool A

Pool B

Pool C

1. MLR
2. 51Cr release assay
3. Spleen colony assay (CFU-S)

Spleen cells

Day 15

1. MLR
2. 51Cr release assay
3. Transplant for GVHD assay
4. Transplant into irradiated isogeneic recipients

Fig. 6. Experimental protocol for the study of the regeneration of immunocompetent cells in irradiated CS7BL/6J mice after transplantation of fractionated isogeneic bone marrow cells.

ions in each region to give three pools of cells. Cells in pool A came from fractions having a density range of 1.05–1.059 g/cm³. Therefore, this pool is enriched for CFU-S and contains few, if any, MLR-responsive cells. Pool B contains cells from fractions having a density range of 1.06–1.07 g/cm³. It contains a mixture of MLR-responsive cells and CFU-S. Pool C contains cells coming from fractions with a density range of 1.071–1.079 g/cm³, and therefore contains some MLR-responsive cells but very few CFU-S. As a base line, samples of cells from each pool were assayed for the presence of CFU-S and cells capable of responding in the MLR and the 51Cr release assays before transplantation (day 0). Then samples of 4 × 10⁶ cells from each pool were injected into different groups of irradiated isogeneic recipients. 15 days later, the recipients' spleens were removed and tested for the presence of cells capable of responding in the MLR and 51Cr release assays as well as for cells with the capacity to induce acute GVHD in allogeneic recipients (DBA/2J).
The inoculum of cells from pool A contains no MLR-responsive cells or cells capable of lysing specific target cells; it contains the majority of CFU-S (Fig. 7). The inoculum of cells from pool B contains most of the MLR-responsive cells and some CFU-S, while the inoculum from pool C contains few MLR-responsive cells and very few CFU-S. However, assays of the spleens from each group of recipients 15 days after transplantation for MLR-responsive cells and cells active in the $^{51}$Cr release assay showed that the greatest activity in both of these assays came from mice which had been transplanted with cells from pool A (Fig. 7, Table III). Furthermore, spleen cells from recipients of bone marrow cells in pool A were also active in producing acute GVHD (Fig. 8).

![Fig. 7](image-url)  

**Fig. 7.** The results of a typical experiment using the protocol outlined in Fig. 6. The number of CFU-S and the MLR activity (net counts per minute) in the inoculum of $4 \times 10^6$ transplanted C57BL/6J bone marrow cells from each pool before transplantation (day 0) is represented by the dotted and open columns, respectively. The MLR activity recovered per spleen in irradiated C57BL/6J recipients 15 days after transplantation is represented by the shaded column. Spleen cells from irradiated C57BL/6J mice not given a transplant of bone marrow assayed 15 days after irradiation did not produce a detectable MLR response.

Spleen cells obtained from recipients of bone marrow cells from pool B were also active in all three assays, but the spleen cells from recipients of bone marrow from pool C showed little activity. Therefore, these results indicate that region A, although not containing any detectable cells responding in the MLR or giving rise to effector cells before transplantation, nevertheless contains a class of cells which develop into immunocompetent cells after transplantation into isogeneic recipients.

**Nature of the Immunocompetent Cells Present in the Spleens of Mice Transplanted with Fractionated Bone Marrow.**—Since the cells obtained from the spleens of recipients of mice transplanted with bone marrow cells from the light-density region of the gradient are capable of responding in the MLR and giving rise to GVHD, it is likely that they are T cells. Therefore, we deter-
mained the effect of anti-\(\theta\) antibody on the spleen cells of these recipients. The results show that treatment with anti-\(\theta\) ascitic fluid and complement markedly reduces the capacity of these cells to respond in the MLR (Table IV). There-

| Cells assayed                                      | \(\%\) Cr release |
|---------------------------------------------------|-----------------|
| Spleen cells of irradiated mice receiving fractionated bone marrow from the density region: |                 |
| 1.05-1.059 g/cm\(^3\)                             | 28              |
| 1.06-1.070 g/cm\(^3\)                             | 18              |
| 1.071-1.079 g/cm\(^3\)                            | 16              |
| Normal spleen cells\(\ddagger\) (control)        | 68              |

\(\ast\) \(6 \times 10^6\) spleen cells from each group of recipients were incubated with \(6 \times 10^6\) irradiated DBA/2J spleen cells for 4 days. Then cells from these cultures were mixed with \(10^6\) \(^{51}\)Cr-labeled DBA/2J mastocytoma cells and incubated for a further 5 h.

\(\ddagger\) Control consists of \(6 \times 10^6\) normal C57BL/6J spleen cells incubated with \(6 \times 10^6\) DBA/2J irradiated spleen cells as above.

![Graph](image)

**Fig. 8.** Survival of irradiated DBA/2J mice receiving only \(10^6\) isogeneic marrow cells \((\triangle--\triangle), or 10^6\) isogeneic marrow cells plus \(10^7\) spleen cells from irradiated C57BL/6J recipients of fractionated C57BL/6J bone marrow from pool A (\(\bullet--\bullet\)), pool B (\(O--O\), and pool C \(\triangle--\triangle\)).

Therefore, we conclude that the immunocompetent cells in these spleens are indeed T cells.

**Effect of Treatment with Anti-\(\theta\) Antibody on the Progenitors of T Cells.**—The capacity of cell suspensions from the light-density region of fractionated bone marrow cells to give rise to immunocompetent cells in irradiated recipients
can be interpreted in two ways. Either this suspension of cells is contaminated by T cells capable of giving rise to more T cells in the recipients, or it contains progenitors of T cells. To exclude the possibility of contamination by T cells, a suspension of cells obtained from the light-density region of the gradient (density range 1.05–1.059 g/cm³) was treated with anti-θ ascitic fluid and complement before transplantation into isogeneic irradiated recipients. A control group consisted of mice receiving cells from the same density region not treated before transplantation. 15 days later, the spleen cells from each group of recipients were tested for the presence of MLR-responsive cells as described previously. We found that treatment of the transplanted marrow cells with anti-θ in the presence of complement had no effect on the subsequent development of cells capable of responding in the MLR (Table V). This result makes it unlikely that cell suspensions from the light-density regions were contaminated by

TABLE IV

| Treatment                  | Net cpm ± 1 SE |
|----------------------------|---------------|
|                            | Exp. 1        | Exp. 2        |
| Nil                        | 1,420 ± 195   | 2,265 ± 145   |
| Anti-θ‡ alone              | 815 ± 210     | 2,680 ± 695   |
| Complement alone           | 1,245 ± 400   | 3,130 ± 1,485 |
| Anti-θ plus complement     | 58 ± 110      | 410 ± 195     |

* See text for experimental protocol. 4 × 10⁶ C57BL/6J recipient spleen cells treated as indicated, and then incubated with 4 × 10⁶ heavily irradiated DBA/2J spleen cells.
‡ A 1 in 2 dilution of anti-θ ascitic fluid.

TABLE V

| Experiment no. | Treatment‡ | Net cpm/4 × 10⁶ spleen cells (±1 SE) | Colonies/spleen |
|----------------|------------|-------------------------------------|-----------------|
| 1              | Nil        | 5,565 ± 2,345                       | 19 ± 1.8        |
|                | Anti-θ (1:5)‡ plus complement | 4,395 ± 2,280                       | 16 ± 2.6        |
| 2              | Nil        | 4,850 ± 520                         | 14 ± 2          |
|                | Anti-θ (1:2) plus complement | 3,320 ± 395                         | 14 ± 1          |

* See text for experimental protocol. The MLR assay was done using recipients' spleen cells obtained 15 days after transplantation of fractionated marrow from the density region 1.05–1.059 g/cm³ (pool A).
‡ As a positive control, normal spleen cells were treated with anti-θ plus complement under the same conditions. The response in the MLR by these cells was 4% of the response in the untreated controls.
§ Dilution of anti-θ ascitic fluid. Guinea pig serum was used as a source of complement.
T cells capable of giving rise to more T cells in the recipients. Therefore, we suggest that the light-density region of the gradient contains a class of cells, not sensitive to anti-Î³ antibody, that are progenitors of T cells. We will refer to these progenitors as PT cells.

Density Profile for PT Cells.—To determine the density profile for PT cells, C57BL/6J bone marrow was subjected to density centrifugation. A sample of $4 \times 10^6$ cells from each fraction was injected into a group of irradiated (800 rad) isogenic recipients. Fractions in the light-density region yielded an insufficient number of cells to assay each fraction independently. Therefore, these fractions were pooled to give two large fractions; the first one included cells in the density region 1.045–1.05 g/cm$^3$, the second included cells in the region 1.051–1.055 g/cm$^3$. 15 days after transplantation, spleen cells from each group of recipients were tested for activity in the MLR. The results are expressed as counts per minute per spleen (Fig. 9). For comparison the density profiles for CFU-S and MLR in C57BL/6J bone marrow are shown in the same figure.

The results show that the density profile for PT cells is different from those of T cells and CFU-S. PT cells (modal density of 1.064 g/cm$^3$) are lighter in density than most T cells but heavier than most CFU-S. This peak of PT cell activity was reproduced in three experiments whereas the other two apparent peaks of PT cell activity present in the light and dense regions of the gradient as shown in Fig. 9 were not seen in the other two experiments.

DISCUSSION

Transplantation of either mouse bone marrow or spleen cells into allogeneic recipients results in GVHD (15, 16). In the case of spleen cells, it is generally
accepted that the disease is produced by T cells transferred in the inoculum (17, 18). However, in the case of bone marrow transplants, the cellular basis for development of GVHD in the recipients is uncertain. One possibility is that normal mouse bone marrow contains sufficient numbers of T cells capable of producing GVHD. Another possibility is that T cells capable of reacting against allogeneic tissue antigens are developed in the recipient from progenitors present in the bone marrow transplant. In our experiments, we attempted to distinguish between these two alternatives by using buoyant density centrifugation as the means of obtaining enriched populations of cells. We found that normal mouse marrow contains a readily detectable population of cells capable of responding in the MLR and of producing acute GVHD in irradiated allogeneic recipients. These cells band at a modal density of 1.069 g/cm³. Since the capacity of these cells to respond in the MLR is inhibited by the cytotoxic action of anti-θ antibody and complement, we conclude that they are T cells.

Having determined the position on density gradients of T cells present in mouse bone marrow cell suspensions, we then proceeded to test cells obtained from various regions of the gradient for their capacity to give rise to T cells in irradiated recipients. We found that cells obtained from a light-density region of the gradient (1.050–1.059 g/cm³) gave rise to immunocompetent cells in the spleens of irradiated isogeneic recipients within 15 days of transplantation. The cells in the spleens of the recipients were capable of responding in the MLR and of producing acute GVHD when transplanted into allogeneic irradiated recipients. Furthermore, the capacity of these cells to respond in the MLR was inhibited by pretreatment with anti-θ antibody and complement. To exclude the possibility that the bone marrow cell suspension obtained from the light-density region of the gradient contained sufficient T cells capable of repopulating the immunological system of the irradiated recipients, we repeated the above experiment after pretreatment of the cell suspension with anti-θ antibody and complement. A control group of irradiated isogeneic recipients received cells from a similar suspension not pretreated with anti-θ antibody and complement. Spleen cells from these two groups of recipients had an equal capacity to respond in the MLR. Therefore, we can conclude that the T cells found 15 days after transplantation in the recipients’ spleens must have been derived from progenitor cells that are not sensitive to the cytotoxic action of anti-θ antibody. Therefore, these progenitor cells are not identical with T cells.

The identity of PT cells is still unclear. Since we do not have a dose-response relationship for PT cells, precise determination of their activity profile is not possible. However, the preliminary results indicate that the PT cell activity profile shown in Fig. 9 appears to be different from that of T cells and of CFU-S. PT cells are found mainly in the medium-density region of the gradient (modal density of 1.064 g/cm³). Thus, they are lighter in density than T cells and heavier than CFU-S. Whereas T cells can react to foreign histocompatibility antigens, PT cells do not, but can give rise to cells with the properties of T
cells. Furthermore, treatment with anti-\(\theta\) antibody and complement inhibits T-cell function while having no effect on PT cells. The relationship between PT cells and CFU-S is not clear at the present time. PT cells may represent a subpopulation of CFU-S, or alternatively they may be related to each other in a parent-progeny relationship.

Lafleur et al. (19) have identified a progenitor for bone marrow (B) cells in mouse bone marrow (PB cell) and defined it as a cell that does not interact with sheep erythrocytes and T cells to produce a significant antibody response 8 days later. However, this class of cells can differentiate to a cell population with properties similar to that of bone marrow cells. In our experiments, the density measurements for PT cells appear to be similar to those obtained by Lafleur et al. for PB cells, both having a peak modal density of 1.064 g/cm\(^3\). This correlation may be interpreted as meaning that these two progenitors are identical.

We believe that our results have a bearing on the use of density centrifugation as a means of removing immunocompetent cells from human bone marrow before transplantation (20). Even if it were possible to discard T cells which might be present in human marrow, the light-density region to be used for transplantation may contain progenitors capable of producing GVHD after differentiation in the host. On the other hand, these same progenitors may give rise to a T cell population in the recipient necessary for normal immunological defenses. Up to the present time, the results of bone marrow transplantation indicate that the most successful grafts have been in HL-A-identical and MLR-negative siblings (21). The results of marrow transplantation in other donor-recipient combinations have not been as successful. We suggest that the reason for these failures may be explained by the presence, in the grafts, of progenitor cells capable of differentiating into cells with graft-vs.-host potential.

SUMMARY

An experimental model system was developed to study the differentiation of thymus-derived (T) cells from progenitors in bone marrow. In this system transplantation of bone marrow cells depleted of T cells gave rise to T cells in the spleens of irradiated recipients within 15 days of transplantation. Thus, normal bone marrow contains a class of cells that are progenitors of T cells (PT cells). PT cells are different from T cells since PT cells are incapable of responding to alloantigen, are \(\theta\) resistant, and band in a lighter density region than do T cells. The density profile of PT cells is different from that of hematopoietic stem cells (CFU-S); PT cells band in a denser region of the gradient than do CFU-S.

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