Thymus-Marrow Cell Combinations. Synergism in Antibody Production.* (31353)

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(Introduced by D. W. Talmage)
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The source of potentially immunocompetent cells and the regulatory mechanisms involved in their maturation are unsolved problems of great interest. Recently considerable evidence has accumulated indicating that the thymus plays an important role in lymphocytopoiesis and in the development and maintenance of the immune system(1). Although small amounts of antibody are synthesized within the intact thymus(2), a more important role for this structure may be to provide potentially competent lymphoid cells which migrate to other lymphoid structures, such as the spleen and lymph nodes, there to differentiate into immunologically active cells(3). Data also exist showing that the thymus may produce a diffusible hormone-like product, influencing the multiplication and maturation of lymphoid cells in the peripheral lymphoid tissues(4). These two mechanisms are by no means mutually exclusive.

The experiments reported here sought to test for the esistence of potentially immunocompetent cells in the thymus, according to the method recently described for spleen cells by Playfair, Papermaster, and Cole(5). This method permits the study of *potentially* immunocompetent cells by demonstrating discrete clones of active, antibody-producing cells arising from a unit number of non-sensitized progenitors which are transferred to irradiated hosts and stimulated with antigen. In the course of these experiments, we found that combinations of normal thymus and bone marrow cells were far more active in producing hemolysins than cells of either type alone. Graft vs. host activity of cells was eliminated since the donor and recipient cells were of the same inbred strain.

Methods. Nine-to twelve-week-old LAF_1 or CBA/J recipient male mice were given a single exposure to 650-750 r (in air) 250 kvp x-rays (30 ma, 0.5 mm CU, 1.0 mm Al, 100 cm) followed by intravenous injection of syngeneic cells (spleen, thymus, marrow or thymus plus marrow). Donor spleen or thymus cell suspensions were obtained by forcing tissue through a stainless steel screen into cold Saline F. Marrow was extruded from femurs by forcing saline through with a syringe after cutting off the epiphyses, and suspensions were made by forcing marrow clumps through a 26 gauge needle. Cells were washed in cold Saline F and counted in a hemocytometer. Concentrations were adjusted so that each animal received the desired number of cells in 0.5 ml of Saline F. When mice received both thymus and narrow cells, the 2 cell populations were combined shortly before injection in a total volume of 0.5 ml. All recipients were injected within 2 hours following irradiation.

In the first group of experiments, the mice were injected iv with 0.2 ml of a 10% suspension of washed sheep erythrocytes on the first day following irradiation. Five days following irradiation, the mice were killed and their spleens removed, cut into fragments, and plated after the manner of Playfair *et al*(5), except that red pulp was not trimmed from the fragments. After 2 hours, incubation at 37°C, 2 ml of 33% guinea pig complement was added to each plate. The plates were incubated for 30 minutes more, fixed by pouring 10% neutral formalin over the surface, then coded and read as unknowns and scored by 2 methods. The experimental design is illustrated in Fig. 1.

The spleen fragments with significant surrounding hemolysis ("active pieces") were not randomly distributed but tended to occur in clusters or "active areas." Playfair *et al*(5) have shown that an active area is probably derived from a unit number of precursor cells, but when there are many active

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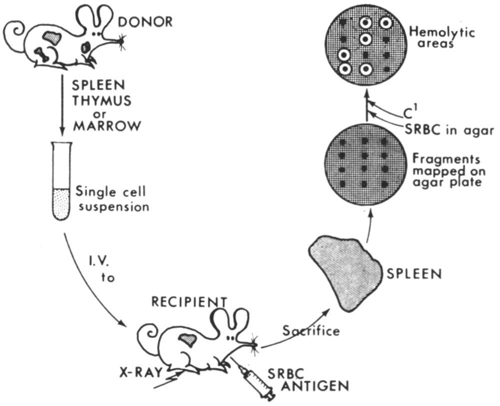


FIG. 1. Experimental design.

pieces, the delineation of discrete active areas is difficult since they tend to overlap. For this reason, the percentage of spleen pieces with hemolysis was also calculated and designated "specific activity." This was found to be linearly related to the number of active areas and permitted quantitative scoring of a larger range of activities. In evaluation of the data ± two standard errors were considered to be the 95% confidence interval.

To determine the percentage of cells reaching recipient spleens, separate suspensions of CBA spleen and thymus cells were incubated for 6½ hours with H^3-thymidine. The cells were washed and the percent labelling determined by autoradiographs of aliquots. Irradiated recipients were injected iv with 10^6 thymus or spleen cells and were sacrificed one hour later. The recipient spleens were made into single cell suspensions and the total number of cells counted. Smears of measured aliquots were made and the number of labelled cells determined by autoradiography. The total number of labelled cells in the spleen was calculated to be 6.5% of those labelled spleen cells injected, and 2.9% of those labelled thymus cells injected. Assuming that the distribution of labelled cells reflected the distribution of all donor cells, roughly twice as many spleen as thymus cells reached the recipient spleens.

Results. Table I shows the results of the first group of experiments using normal and immune spleen and thymus cells. When 5 × 10^6 spleen cells from non-immunized CBA/J donors were injected into irradiated syngeneic recipients, the mean number of active areas in the spleen 5 days later was found to be 2.5 times that found when 2 × 10^6 spleen cells were injected. Approximately 2.2 × 10^6 cells produced one active area in both cases. Five hundred thousand spleen cells from donors injected 3 days previously with sheep red cells (labelled immune) produced as many active areas as two million non-immune cells. Thymus cells from normal and immunized donors did not produce significantly more active areas than controls given no cells.

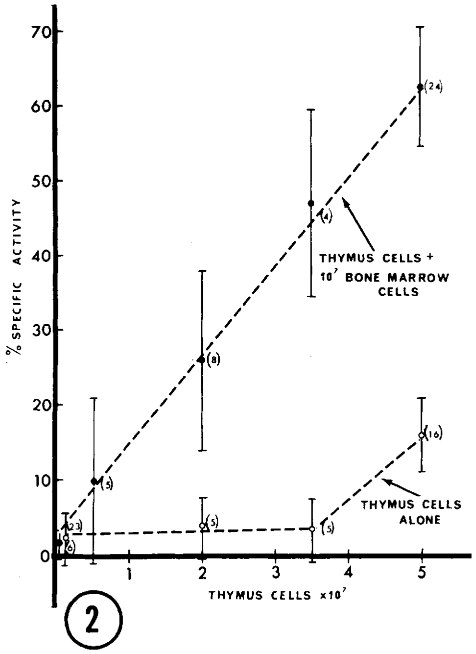
In the next group of experiments the x-rayed recipients received injections of antigen on days 1 and 4 and were sacrificed on day 8 following irradiation (Table II). Some animals were given 10^7 sygeneic marrow cells either alone or together with thymus cells. Spleens of mice receiving 5 × 10^7 thymus cells plus 10^7 marrow cells had more and strikingly larger zones of lysis than animals receiving either cell type alone. Marrow alone showed no activity above background, but thymus alone at 8 days showed slight activity.

LAF_1/J mice were used in all subsequent experiments because of their greater resistance to x-rays. Fig. 2 presents pooled data from 6 experiments showing specific activity as related to the dose of thymus cells. When mice received 10^7 marrow cells (upper line), the response curve was linear at least through 5 × 10^7 thymus cells, with 5.5 × 10^6 cells producing one active area and 10% specific

TABLE I. Production of Active Areas of Hemolysis in spleens of CBA Mice Which Were Irradiated and Injected with Spleen or Thymus Cells on Day 0, Given Sheep Erythrocytes on Day 1 and Sacrificed on Day 5.

Cells injected	No. of cells (× 10^6)	Active areas per spleen	Mean
Normal spleen	2	00111112	.87
” ”	5	111122222222 334444	2.3
Immune spleen	.5	00113	.80
” ”	5	****	*
Normal thymus	20	00000002	.25
” ”	50	0000	.0
” ”	90	00011	.40
Immune thymus	100	000	.0
Control	0	0000000000 0000001111	.2

* Too many active areas to count accurately.



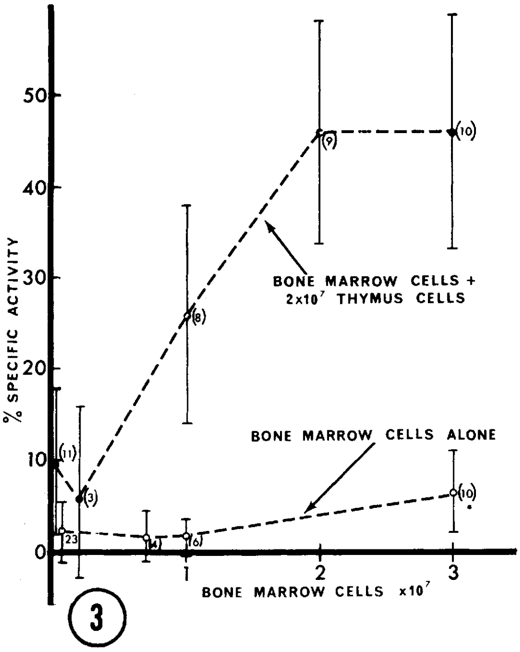


FIG. 2. Percent specific hemolytic activity of recipient spleen fragments are related to number of thymus or thymus plus 10^7 marrow cells injected. Numbers in parentheses represent numbers of mice for each data point. Range indicates ± two standard errors.

FIG. 3. Percent specific hemolytic activity of recipient spleen fragments as related to number of marrow or marrow plus 2×10^7 thymus cells injected. Numbers in parentheses represent numbers of mice for each data point. Range indicates ± two standard errors.

activity. Thymus cells alone (lower line) were much less effective and approximately 4.3×10^7 cells were required to produce one active area (10% specific activity).

In the reciprocal experiment (Fig. 3), the hemolytic response was evaluated as a function of the number of marrow cells injected. In the groups of mice receiving varying numbers of marrow cells plus 2×10^7 thymus cells (upper line), the response was linear between 2×10^6 and 2×10^7 marrow cells, but leveled out with higher doses of marrow. Recipients of marrow cells alone (lower line) were never significantly more active than controls given no cells.

Discussion. The results confirm the work

TABLE II. Hemolytic Activity in Spleens of CBA Mice Which Were Irradiated and Injected with Spleen, Thymus, and/or Marrow Cells on Day 0, Given Sheep Erythrocytes (iv on Day 1 and ip on Day 4) and Sacrificed on Day 8.

Cells injected	No. of cells ($\times 10^6$)	Active areas per spleen	Mean active areas	Mean specific activity (% ± S.E.)
Thymus	35-50	00011111 2333	1.3	12.3 ± 3.9
Marrow	10	0000000000 01111122	.5	1.6 ± .5
Thymus + marrow	50 10	1334** ******	>3.0	70.7 ± 3.3
Spleen	5	12334* *****	>3.0	66.1 ± 9.0
None		000000000 111112	.5	7.4 ± 2.9

* Too many active areas to count accurately.

of Playfair *et al*(5) by showing the production of clusters of antibody-producing cells in the spleens of irradiated syngeneic mice given normal spleen cells and then challenged with antigen. Over the range tested, the mean number of active areas was directly proportional to the number of cells injected. Five × 10^6 spleen cells produced more active areas in 8-day experiments than in 5-day experiments. Since 2.2 × 10^6 normal spleen cells produced one active area, and presumably contain one precursor cell, and since 6.5% of tranferred spleen cells (labelled *in vitro*) appear in the recipient spleen, then one precursor is contained in 143,000 normal spleen cells. (Although it is possible that more cells may eventually localize in the spleen following temporary trapping in the lungs, the value presented here agrees quite well with the 4% reported by Playfair *et al*(5) 24 hours following transfer.) Since a normal mouse spleen contains about 10^8 nucleated cells, it would contain 700 precursor cells. This is considerably more than the 50 precursors estimated by Makinodan and Albright(6) or than the 100 precursors estimated by Jerne *et al*(7).

The thymus does not appear to contain similar potentially immunocompetent cells since thymus cells from normal or immunized donors did not produce significant hemolytic activity in recipient spleens at 5 days. At 8 days there was a small amount of activity in recipients of thymus cells, but none in recipients of marrow cells. The combination of thymus and marrow cells, however, produced more active areas and greater specific activity than can be accounted for by simple summation of the activities of the two donor populations.

The simplest interpretation is that one cell population contains cells capable of making antibody ("effector cells"), but only in the presence of cells from the other population ("auxiliary cells"). These data do not establish which cell suspension contains either effector or auxiliary cells nor how these cells interact(8).

Other transfer experiments have shown antibody production by thymus cells from immunized donors, but the cells have generally been transferred many weeks after donor immunization began(9). The known antibody-producing activity of marrow from immunized donors(10) together with evidence that marrow cells migrate through the thymus(11) make it possible that the immunocompetence of transferred thymus from immunized donors is due to the presence of marrow-derived cells within the thymus. A growing body of data shows that the immunocompetence of marrow cells depends upon the presence of the thymus(12). On the basis of all these data, we feel that it is most likely that the effector cell in our experiments is marrow-derived, and that the thymus provides the auxiliary cells.

Summary. Suspensions containing normal thymus, spleen, or marrow cells were injected into irradiated syngeneic mice which were subsequently given antigen. Normal spleen cells produced discrete areas of antibody production in recipient spleens, and the number of areas was proportional to the number of donor cells. Mice receiving both marrow and thymus cells produced more centers of hemolytic activity in their spleens than mice receiving cells of either type alone. Normal and immunized thymus cells produced little or no hemolytic activity, and normal marrow was also inactive.

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Red Cell Charge as Affected by Low Viscosity Dextran.* (31354)

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Electrical charge as a factor in the mutual repulsion of red blood cells was first suggested by Jevons in 1870(1). After the introduction of a microcataphoretic cell by Northrop and Kunitz(2), actual measurements became possible. Improvements in the technique were developed by Abramson(3) and by Moyer(4). Bernstein *et al* measured the cellular charge as affected by *in vitro* mixing of blood and low molecular weight dextran(5). The following study includes determinations of the effects of trauma and of low viscosity dextran† (LVD) on the surface charge of erythrocytes by means of a previously developed electrometric method (6,7).

Experimental procedure. Electrometric studies were made on the erythrocytes of 41 patients from the surgical wards of Cook County Hospital. Eleven untreated control patients who were awaiting elective cosmetic surgery were considered to be normal from the point of view of their circulation. Five other patients who had recently undergone elective herniorrhaphy were also studied. They were infused with one liter of physiologic saline at the rate of 5 ml per min before and after which electrometric determinations were made.

Thirty patients received intravenous infusions of LVD in the dose of 1.4 g per kg body weight at the rate of 5 ml per minute and the cellular charge was measured on samples obtained 30 minutes after termination of the infusion. Eleven of the patients were normal controls. Fourteen patients who had undergone either abdominal or head and neck surgery were infused 2 to 4 hours after operation in a similar manner. In addition, the electrical charge of erythrocytes of 11 patients were either septic or hypovolemic shock were also studied electrometrically. LVD then was administered to 5 patients of this group and electrical charge again was measured.

Electrometric Method. Ten ml blood samles were obtained by antecubital venipuncture. Heparin (10 units) was added to each sample. The samples were collected in plastic test tubes and were immediately centrifuged in a refrigerated centrifuge at 2000 *g* for 20 minutes. A small perforation was made in the bottom of the tube, and about 2 ml of packed cells were drained by gravity into a second test tube containing 1 ml of 0.15 M NaCl buffered at pH 7.4. This sample was centrifuged under the same conditions.

The electrometric method, as previously described for connective tissues(8), epidermis (9) and ascites cells(10 was adapted to the measurement of red cell charge.

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† Low viscosity dextran, Rheomacrodex®, was kindly supplied as a 10% solution in isotonic saline (Batch No. 4847), by the Pharmacia Laboratories, Uppsala, Sweden. Low viscosity dextran has an average molecular weight of 41,000 with an intrinsic viscosity of 0.19. For simplicity, low viscosity dextran is referred to as LVD.