SYNERGISM OF THYMUS AND BONE MARROW IN THE PRODUCTION OF GRAFT-VERSUS-HOST SPLENOMEGALY IN X-IRRADIATED HOSTS*, †

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Thymus cells and bone marrow cells have been shown to interact synergistically in the humoral immune response to sheep erythrocytes. If thymus and bone marrow cells are injected together into heavily X-irradiated hosts, there is approximately a 10-fold increase in the number of antibody-forming cells compared to that seen after injection of either thymus cells or bone marrow cells alone (1, 2). Attempts to demonstrate such an interaction of thymus cells and bone marrow cells in a cellular immune response, the graft-versus-host reaction, have been unsuccessful (3, 4). However, these experiments involving the graft-versus-host reaction were carried out in unirradiated hosts, and therefore are not strictly comparable to the sheep erythrocyte experiments, in which hosts received at least 650 R X-irradiation (1, 2). In the unirradiated host, functional activities of injected donor cells may be masked by the similar activities of host cells, and in the irradiated host, normal functional activities of donor cells may be altered by the environment which promotes rapid regenerative proliferation. Therefore, in order to carry out experiments on the graft-versus-host reaction which would be comparable to those involving the humoral immune response, we have assessed the capacity of cell suspensions from thymus and bone marrow to produce graft-versus-host splenomegaly in X-irradiated hosts. We report here that cell suspensions of thymus and bone marrow interact synergistically in the production of graft-versus-host sple-
nomegaly in X-irradiated hosts. The mechanism of synergism is different however, in the production of splenomegaly than in the production of antibody to sheep erythrocytes.

Materials and Methods

Mice.--Highly inbred mice of the A and C57Bl/1 strains and LAF1 mice resulting from the cross between these strains were used for these experiments. Young 7- to 10-day-old mice of the LAF1 strain were used as recipients of cell injections. Donors of spleen and bone marrow cells suspensions were 45- to 90-day-old A and LAF1 mice. Recipients were left with their mothers except during experimental manipulations.

X-Irradiation.--X-irradiated animals (7–10 days of age) were exposed to 300 or 500 R 24 hr before cell injection. Irradiation factors were: 250 kvp, 15 ma, filters 0.5 mm Cu and 1 mm Al, half-value layer (HVL) 1.49 mm Cu, target skin distance (TSD) 40 inches, dose rate 27.8 R per min. No deaths occurred as a result of radiation exposure during the course of these experiments.

Cell Suspensions.--Donor mice were sacrificed by cervical dislocation. Thymi were surgically removed, placed in Medium 199, sliced into six to eight pieces, and the cells gently dispensed in a loosely fitting Potter-Elvehjem glass tissue homogenizer. The cell suspension was poured from the homogenizer into a Petri dish, and after passage of the suspension through a 25 gauge needle, the cells were counted and collected into 1 ml syringes for injection. Bone marrow suspensions were prepared from donor femurs by the method of Ainsworth and Larsen (5). A small hole was made in the intercondylar fossa of the femur with a 25 gauge needle; the marrow was discharged through this hole by applying pressure to a needle inserted into the marrow cavity at the opposite end of the femur between the greater trochanter and the head. Medium 199 containing 0.5 unit of heparin per ml was used for marrow collection. After collection, the marrow suspension was filtered through fine mesh nylon (pore size of 0.3 mm) and the cells collected and counted.

Assay of Immunologic Competence.--The graft-versus-host assay described by Simonsen et al. (6) was used. This assay measures splenic enlargement in F1 hybrid recipients of immunologically competent cells from one parental strain. Since the F1 hybrid mice are genetically tolerant of cells derived from either parental strain, a host-versus-graft immunologic reaction is not a factor in the assay system. In a non-immunologic sense, however, a host-versus-graft reaction is present, since the splenomegaly is due mostly to large numbers of mononuclear cells of host origin (7, 8). Strictly speaking, therefore, we are measuring the capacity of the donor cells to elicit a hyperplastic host response.

The hosts were 7–10 day old LAF1 hybrid mice. For experiments in which cell suspensions from two sources were used, the cell suspensions were injected intraperitoneally, separately. Bone marrow cell suspensions were always administered within 90 min after thymus or spleen cell suspensions. Intraperitoneal injections were performed under ether anesthesia. In the experiments involving host irradiation, the irradiation was given 24 hr before cell injection. 8 days after injection of cell suspensions (9 days postirradiation in irradiation experiments), the animals were sacrificed, their body and spleen weights measured, and the relative spleen weight (ratio of spleen weight to body weight) was determined for each animal. For experiments in which hosts received no X-irradiation, the spleen index was calculated for each injected animal by dividing its relative spleen weight by the mean relative spleen weight of non-injected littermate control animals.

Several experimental results indicate that it is an immunologic attack by donor cells against the host which initiates the host splenomegaly response. For example, cells from donors immunized against host antigens cause an increase in splenomegaly (9), and cells from donors tolerant of host antigens do not elicit splenomegaly (10). Lymphoid cells from donors which are
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genetically incapable of responding to host antigens do not elicit splenomegaly. Thymus cells, while possessing considerable less immunologic competence than cells from lymph node or spleen, also elicit graft-versus-host splenomegaly (11-13). As with lymphoid cells from other sources, thymus cell suspensions from an immune animal elicit increased splenomegaly in a host to which they are immune (14).

Experimental Plan.—Using the graft-versus-host splenomegaly assay as described, five categories of experiments were performed:
(a) Unirradiated LAF1 hosts were injected with parental strain thymus and/or parental strain bone marrow in an attempt to demonstrate an interaction of these cell populations.
(b) Irradiated LAF1 hosts were injected with either parental strain spleen cells or syngeneic spleen cells to demonstrate the effect of host irradiation on the development of graft-versus-host splenomegaly.
(c) Irradiated LAF1 hosts were injected with parental strain spleen cells and syngeneic bone marrow cells in an attempt to reconstitute the graft-versus-host splenomegaly response.
(d) Irradiated LAF1 hosts were injected with parental strain thymus and/or parental strain bone marrow in an attempt to demonstrate synergism in the production of graft-versus-host splenomegaly.
(e) Irradiated LAF1 hosts were injected with syngeneic or parental strain thymus in addition to either syngeneic or parental strain bone marrow in an attempt to dissect the immunological aspects of the thymus-marrow synergism from the nonimmunological aspects.

RESULTS

Parental Strain Thymus and Bone Marrow Cells Do Not Interact to Produce Splenomegaly in Unirradiated LAF1 Hosts.—In order to determine the suitability of these LAF1 hybrids for estimation of splenomegaly produced by A strain thymus cells, $40 \times 10^6$, $20 \times 10^6$, and $10 \times 10^6$ thymus cells from A strain donors were injected into 7-10-day-old LAF1 hybrid recipients (Table I). The spleen index results, obtained 8 days after injection, indicate that $40 \times 10^6$ cells produce greater splenomegaly than $20 \times 10^6$ cells ($P < 0.01$), and similarly that $20 \times 10^6$ cells produce greater splenomegaly than $10 \times 10^6$ cells ($P < 0.05$). Thymus cells from LAF1 donors ($40 \times 10^6$) produce no splenomegaly, ruling out the possibility that nonspecific factors associated with the thymus cell suspension might contribute significantly to splenomegaly.

To determine if an interaction could be demonstrated between thymus cells and bone marrow cells in unirradiated hosts, individual litters of at least eight animals were divided into four groups of at least two animals each and injected with cells from A strain donors as follows: one group injected with $20 \times 10^6$ thymus cells, one group injected with $20 \times 10^6$ bone marrow cells, one group injected with cells from both sources, and one group which was not injected with cells. The results, shown in Table II, indicate (a) that bone marrow cells alone do not exert graft-versus-host reactivity in this system, and (b) that thymus cells and bone marrow cells together elicit the same amount of splenomegaly that the thymus cells do when injected alone.

Elimination of Graft-Versus-Host Splenomegaly by 500 R Host X-Irradiation.—Young 7- to 10-day old LAF1 mice were exposed to 500 R X-irradiation and
injected 24 hr later with (a) no cells, (b) $10 \times 10^6$ spleen cells from A strain donors, or (c) $10 \times 10^6$ spleen cells from LAF₁ donors. Five or more mice of each group were sacrificed on various days following irradiation, and the relative spleen weights of the mice determined. The results, shown in Fig. 1, indicate that by the second postirradiation day there has been a considerable decrease in relative spleen weight in all three groups. The relative spleen weight is increasing rapidly in the groups receiving irradiation only, or irradiation plus syngeneic spleen cells by the 6th postirradiation day, and it appears that recovery is slightly more rapid in the group receiving syngeneic spleen cells. In the group injected with $10 \times 10^6$ A strain spleen cells, however, in which a graft-versus-host attack is occurring, there is a relative failure of recovery of spleen weight, and in fact by the 9th postirradiation day the relative spleen weight in this group is only about half that of the other two groups.

Graft-versus-host splenomegaly has been shown to be primarily due to the proliferation of host cells (7, 8). The absence of spleen weight recovery in these irradiated mice undergoing a graft-versus-host reaction probably indicates that the host cell population is damaged to such an extent by the combined effects of irradiation and immune attack that host cell proliferation cannot occur. If this were the case, unirradiated cells of host genotype, if supplied to the ir-
radiated hosts, might enable the hosts to respond to the immune attack with the usual splenomegaly. We therefore supplied bone marrow cells from unirradiated donors to the irradiated hosts in an attempt to reconstitute the host proliferative response.

![Graph showing the effect of X-irradiation on graft-versus-host splenomegaly.](image)

**FIG. 1. Effect of 500 R X-Irradiation to the host on graft-versus-host splenomegaly.**

- □-□, X-irradiation only.
- ●-●, X-irradiation and injection of $10 \times 10^6$ spleen cells from LAF1 donors.
- ○-○, X-irradiation and injection of $10 \times 10^6$ A strain spleen cells.

Cell suspensions were injected intraperitoneally 24 hr postirradiation. (Bars at day 9 represent ±1 SE).

**TABLE III**

| Graft-Versus-Host Splenomegaly in 500 R X-Irradiated LAF1 Hosts Injected with Syngeneic Bone Marrow* |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| No. of spleen cells injected and strain of spleen cell donor | No. of LAF1 bone marrow cells injected | No. of hosts | Mean relative spleen weight of hosts (±1 SE) |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| None                                            | $10 \times 10^6$ LAF1                           | 23                                                   | $1.090 \pm 0.037$                                |
| $10 \times 10^6$ A                              | None                                            | 7                                                    | $1.014 \pm 0.084$                                |
| $10 \times 10^6$ A                              | None                                            | 11                                                   | $0.372 \pm 0.018$                                |
| $10 \times 10^6$ A                              | $10 \times 10^6$ A                             | 7                                                    | $0.459 \pm 0.069$                                |
| $2 \times 10^6$ LAF1                            | None                                            | 10                                                   | $0.996 \pm 0.100$                                |
| $2 \times 10^6$ A                               | None                                            | 9                                                    | $1.065 \pm 0.078$                                |
| $2 \times 10^6$ A                               | $10 \times 10^6$ A                             | 14                                                   | $1.436 \pm 0.060$                                |

*Hosts injected 24 hr postirradiation with spleen cells and/or bone marrow cells.

**Reconstitution by LAF1 Bone Marrow Cells of the Graft-Versus-Host Splenomegaly Responses in 500 R X-Irradiated LAF1 Hosts.**—Mice receiving 500 R of X-irradiation were injected 24 hr later either with $2 \times 10^6$ or $10 \times 10^6$ spleen cells from A strain donors. Within 90 min after injection of the spleen...
cells, some of these hosts were given an additional injection of $10 \times 10^6$ bone marrow cells from syngeneic LAF1 donors. Both injections were given by the intraperitoneal route. The mice were sacrificed 8 days after cell injection (9 days postirradiation) and the mean relative spleen weights were calculated. The addition of bone marrow cells did not reconstitute the splenomegaly response in the hosts given $10 \times 10^6$ A strain spleen cells (Table III). On the other hand, hybrid bone marrow, injected along with the smaller number ($2 \times 10^6$) of A strain spleen cells, produced significantly larger relative spleen weights than were seen in the groups receiving only marrow cells, or only spleen cells ($P < 0.01$).

### Table IV

**Assay of Thymus Cells and/or Bone Marrow Cells in LAF1 Hosts Receiving 500 R X-Irradiation 24 Hr Prior to Injection**

| Cell source          | No. of cells injected | Strain of cell donor | No. of hosts | Mean relative spleen weight ($\pm 1 SE$) |
|----------------------|-----------------------|----------------------|--------------|----------------------------------------|
| Thymus               | $20 \times 10^6$      | LAF1                 | 15           | $0.72 \pm 0.052$                       |
| None                 | None                  | —                    | 44           | $0.68 \pm 0.042$                       |
| Thymus               | $20 \times 10^6$      | A                    | 19           | $0.76 \pm 0.072$                       |
|                      |                       |                      |              | $P < 0.01$                             |
| Thymus               | $40 \times 10^6$      | A                    | 12           | $0.49 \pm 0.027$                       |
| Bone marrow          | $20 \times 10^6$      | LAF1                 | 16           | $1.05 \pm 0.037$                       |
| Bone marrow          | $20 \times 10^6$      | A                    | 16           | $1.16 \pm 0.056$                       |
| Bone marrow          | $40 \times 10^6$      | A                    | 8            | $1.25 \pm 0.079$                       |
|                      |                       |                      |              | $P < 0.001$                           |
| Thymus and bone marrow each | $20 \times 10^6$      | A                    | 13           | $1.68 \pm 0.052$                       |

From these results it is apparent that host strain bone marrow cells restore the splenomegaly response if the graft-versus-host reaction is initiated by $2 \times 10^5$ spleen cells, but not if it is initiated by $10 \times 10^6$ spleen cells.

**Synergistic Effect of Parental Strain Thymus and Bone Marrow Cells in the Production of Splenomegaly in Irradiated LAF1 Hosts.**—Thymus and bone marrow cells were assessed in LAF1 mice receiving 500 R X-irradiation. 24 hr postirradiation the mice were injected with thymus cells or bone marrow cells, or both, from adult A strain donors. The results are shown in Table IV. The injection of $20 \times 10^6$ thymus cells, whether from A or LAF1 donors, has no significant effect on the relative spleen weight in comparison to the controls receiving irradiation only. However, the injection of $40 \times 10^6$ thymus cells from A strain donors produces a depression of relative spleen weight, reminiscent of the similar depression obtained using spleen cells from A strain donors. If only bone marrow cells ($20 \times 10^6$ or $40 \times 10^6$) are injected, there is a significant
increase in relative spleen weight. This increase is not graft-versus-host splenomegaly, however, since F1 bone marrows, cells, taken from donors syngeneic with the hosts, produce a similar increase in relative spleen weight.

When the LAF1 hosts were injected with both thymus cells and bone marrow cells from A strain donors, there was a significant increase ($P < 0.001$) in relative spleen weight compared to that obtained by injection of either cell population alone. Cells from thymus and bone marrow thus act synergistically in the production of graft-versus-host splenomegaly in X-irradiated hosts.

### TABLE V

**Analysis of the Reactivity of $20 \times 10^6$ Thymus Cells and $20 \times 10^6$ Bone Marrow Cells Injected into 500 R X-Irradiated LAF1 Recipients**

| Strain of thymus donor | Strain of bone marrow donor | No. of hosts | Relative spleen weights of hosts ($\pm$ SE) | Mean spleen index relative to bone marrow control |
|------------------------|-----------------------------|--------------|------------------------------------------|---------------------------------------------|
| None                   | A                           | 16           | $1.16 \pm 0.056$                        | 1.00                                        |
| A                      | A                           | 13           | $1.68 \pm 0.052$                        | 1.45                                        |
| LAF1                   | A                           | 12           | $1.22 \pm 0.076$                        | 1.05                                        |
| None                   | LAF1                        | 16           | $1.05 \pm 0.037$                        | 1.00                                        |
| A                      | LAF1                        | 11           | $1.70 \pm 0.096$                        | 1.62                                        |
| LAF1                   | LAF1                        | 11           | $1.05 \pm 0.052$                        | 1.00                                        |

### TABLE VI

**Titration of Thymus Cells from A Strain Donors in 500 R X-Irradiated LAF1 Recipients of LAF1 Bone Marrow**

| No. of thymus cells injected | No. of hosts | Spleen index ($\pm$ 1 SE) |
|------------------------------|--------------|---------------------------|
| $1 \times 10^5$              | 9            | $1.08 \pm 0.036$          |
| $2 \times 10^5$              | 11           | $1.28 \pm 0.066$          |
| $4 \times 10^5$              | 12           | $1.36 \pm 0.047$          |
| $8 \times 10^5$              | 10           | $1.47 \pm 0.062$          |

* Bone marrow cells and thymus cells injected 24 hr post-irradiation. All mice received $10 \times 10^6$ bone marrow cells.

† Spleen index calculated with respect to littermate controls receiving irradiation and bone marrow, but no thymus cells.

**Marrow Contribution to the Thymus-Marrow Synergism Is Not Immunologic.**—

We next injected various combinations of thymus and bone marrow cells from A and LAF1 donors in an attempt to separate the immune from the nonimmune components of the splenomegaly. Cells from LAF1 donors were employed since these cells obviously cannot mount an immunologic attack against the syngeneic LAF1 hosts. The results, shown in Table V, indicate that in order to obtain a synergistic interaction, the thymus cells must be derived from A strain donors. Therefore, the thymus cells must react against the allogeneic antigens in the host in order to initiate this thymus-marrow splenomegaly. The bone marrow
cells, on the other hand, may be from either A or LAF1 donors, and they therefore perform a nonimmunologic function.

If the thymus cells truly carry out an immunologic reaction in this synergism, then an increase in spleen index should be obtained by increasing the number of thymus cells injected (12, 13). We therefore, injected various doses of A strain thymus cells along with a standard dose (10 × 10^6) of LAF1 bone marrow cells into the 500 R X-irradiated hosts. The results, shown in Table VI, indicate that the mean spleen index (relative to the irradiated control injected only with bone marrow) increases as the number of injected A strain thymus cells increases.

**DISCUSSION**

The results of these experiments demonstrate that thymus cells and bone marrow cells must both be injected in order to produce graft-versus-host splenomegaly in hosts irradiated with 500 R. The degree of splenomegaly, obtained by injecting both thymus and marrow cells from A strain donors into LAF1 hosts, greatly exceeds that obtained by injecting cells from either thymus or bone marrow alone. Evidence that the function of thymus cells in the production of thymus-marrow splenomegaly is immunologic is provided by the finding that this splenomegaly is caused only by thymus cells which can mount an immunologic attack against the host. Further evidence of the immunologic function of the thymus cells is the finding that an increase in the number of thymus cells causes an increase in thymus-marrow splenomegaly. This is the relationship expected for immunologically competent cells exerting a graft-versus-host reaction (9, 12, 13). The bone marrow cells carry out a nonimmunologic function in this synergism, since marrow cells from F1 hybrids are just as effective as marrow cells from parental strain donors in the interaction with thymus cells.

The interaction of thymus and bone marrow shown here represents a true synergism, since the cooperative action of both cell populations is greater than the sum of the actions of the cell populations taken separately. It is important to note, however, that there is no synergism in the production of the specific immune response itself: thymus cells alone are responsible for the immune attack in these experiments. The synergism applies only to splenomegaly, and splenomegaly in turn depends upon a nonimmunologic proliferative response which in these experiments is supplied by cells from the donor bone marrow.

The thymus-marrow synergism shown here is thus not comparable in mechanism to the synergism seen in the humoral immune response. In the humoral immune response to sheep erythrocytes, the bone marrow-derived cells produce specific antibody, and the addition of thymus cells facilitates this antibody production by the marrow cells (2, 15). In this humoral response, then, cells from both sources are involved in the process by which the immune response
itself is produced; whereas in the production of graft-versus-host splenomegaly
the bone marrow cells add nothing to the immune attack which is carried out
entirely by the thymus cells.

Another line of evidence suggests that thymus cells function quite differently
in reactions of the graft-versus-host type than they do in humoral immune
responses. Experiments in which neonatally thymectomized mice have been
reconstituted by injection of suspensions of allogeneic thymus cells show that
the spleen cells from these reconstituted mice which possess graft-versus-host
reactivity have the histocompatibility characteristics of the donor thymus cells
(16). On the other hand, spleen cells producing hemolysin to sheep erythrocytes
from these mice have the histocompatibility characteristics of the host (17).

Synergism in the production of graft-versus-host splenomegaly by lymphoid
cell populations from different sources has recently been reported by Cantor
et al. (18), but these authors do not report on thymus-marrow combinations.
Their experiments which show, for example, thymus-spleen and thymus-node
synergism, were carried out in (Balb/c × C57)F₁ hosts which were injected
with Balb/c or C57 cell populations. These authors suggest that specific im-
mune reactivity is needed on the part of both donor cell suspensions in order to
elicit splenomegaly; however, their experiments concerning specificity may be
unsatisfactory, since the hosts used for the specificity studies were pure bred
C57 mice, in which synergisms were not demonstrated, whereas the synergistic
interactions were all demonstrated in F₁ hybrid hosts. No data are provided
to indicate whether synergism could or could not have been demonstrated in
the C57 hosts had they been injected with two cell populations competent to
react immunologically against them. The situation in an F₁ hybrid host, which
cannot immunologically reject cells from either parental strain, is quite different
from the situation in a C57 host, in which the genetic capability exists to reject
either F₁ hybrid cells or cells from another purebred strain. It is not ruled out,
then, that one or the other cell population in their experiments may have
exerted its effect by contributing significantly to the host proliferative response.
While lymphoid cells might well interact synergistically in producing the
specific immune response of graft-versus-host attack, this point seems not to
have been established yet.

Our failure to demonstrate an interaction of thymus and bone marrow in the
production of splenomegaly in unirradiated hosts confirms the results of
Stutman and Good (3) and Davis et al. (4). Perhaps the interaction cannot be
demonstrated in an unirradiated host because the proliferative response of the
donor marrow cells is masked by the similar proliferative response which the
host cells carry out.

Also of interest is the finding that the spleen weight recovers very slowly after
X-irradiation in recipients of 40 × 10⁶ parental thymus cells or 10 × 10⁶
parental spleen cells. The explanation of this finding may be that the double
insult of immune attack and X-irradiation has caused a greater impairment of the proliferative capacity of host cells than would be caused by irradiation alone. The graft-versus-host reaction may also prevent donor cells from functioning in the host (19, 20). Our experiments show that host strain bone marrow cells can reconstitute the splenomegaly response in irradiated recipients of small numbers (2 × 10⁵), but not larger numbers (10 × 10⁵), of attacking spleen cells. The failure of the bone marrow cells to reconstitute the host response in recipients of 10 × 10⁶ cells may be due to an inability of the marrow cells to colonize recipients in which the immunologic damage is great.

The present experiments have revealed that the graft-versus-host response of splenomegaly can be dissected into at least two component parts. The first component is the immunologic attack, initiated in these experiments by thymus cells, and the second is the host proliferative response to the immunologic attack. Irradiation has facilitated this dissection by causing a reduction in the host proliferative component, which we have reconstituted by injecting bone marrow cells. We have not identified the cell type from the bone marrow which restores the splenomegaly response to the irradiated host, but it seems likely that the macrophage population may be involved. It is known that macrophages are derived from bone marrow (21, 22), and it is also known that macrophage-like cells, derived from bone marrow, are involved in reactions of delayed hypersensitivity providing a necessary but immunologically nonspecific component of the reaction (23).

Finally, it should be pointed out that there are problems associated with the use of the graft-versus-host assay to search for the interactions of donor cell populations. One problem is that the host supplies not only the environment for the donor cells, but also the histocompatibility antigen to which the donor cells respond and the cells whose proliferative response must be assessed. Such complexity does not accompany the assessment of a humoral immune response to sheep erythrocytes carried out in an irradiated host (1, 2), since the host here supplies only the environment for the donor cells, not the antigen or the cells whose proliferation must be assessed. Furthermore, during a graft-versus-host reaction, cells of donor (graft) genotype, including hematopoietic stem cells (20), may be functionally impaired, possibly as a result of a “bystander effect” in which cells which do not suffer from direct immune attack are nonetheless damaged (24, 25). Therefore, the possibility cannot be completely ruled out that the presence of an ongoing graft-versus-host reaction has somehow prevented the donor marrow cells from participating in a specific immune reaction which might otherwise have been demonstrable.

SUMMARY

Graft-versus-host splenomegaly may be elicited from 500 R X-irradiated F₁ hybrid hosts if the hosts are injected with bone marrow cells and thymus cells...
from parental strain donors. Cells from thymus only or bone marrow only will not elicit graft-versus-host splenomegaly in these hosts. In this requirement for cells from both sources, the bone marrow cells play a nonimmunologic, proliferative role in the splenomegaly, and the thymus cells carry out the immunologic attack. Thus the mechanism of this synergism is quite different from that reported for the humoral immune response to sheep erythrocytes in which both thymus and marrow interact in the production of the specific immunologic response itself.

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