

CONTRIBUTION OF A THYMIC HUMORAL FACTOR TO THE
DEVELOPMENT OF AN IMMUNOLOGICALLY COMPETENT
POPULATION FROM CELLS OF MOUSE BONE MARROW

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Several lines of investigation suggest that the small lymphocytes considered responsible for immune reactions of the cell-mediated type (1) can differentiate from immune-incompetent precursor cells which arise in the bone marrow. Although the bone marrow population contains cells which are morphologically similar to lymphocytes (2), in rodents this population manifests only a minimal degree of immunological activity as measured by the ability to initiate a *graft-versus-host* type of reaction in appropriate hosts (3-5). There are indications, however, that precursors of the immunologically active cell population are located within the bone marrow compartment of adult animals. Transfer of parental bone marrow cells to newborn hybrid rats subsequently resulted in *graft-versus-host*-competent lymphocytes of donor characteristics within the thoracic duct lymph of the recipients, while in a similar transfer system, precursor cells were not detected in an inoculum of thoracic duct cells (3). Transfused syngeneic bone marrow cells can sustain life and lead to repopulation of the lymphoid tissues in lethally irradiated recipients whereas inocula from lymph nodes, thymus, or thoracic duct seem to lack the necessary undifferentiated cells (6-8). Immunologic function is eventually restored in lethally irradiated mice receiving bone marrow cells, but in the absence of the thymus, immunocompetence is not recovered (9). Thus, differentiation of these incompetent precursors of bone marrow origin to cells which are able to carry out immune responses appears in some way dependent upon the thymus. In other experiments, a pathway of migration of bone marrow cells to the thymus is indicated by detection of chromosome-labeled bone marrow inocula within thymus tissue of lethally irradiated recipients (8), within normal thymus tissue grafted into thymectomized irradiated mice (10), and within the thymus of normal intact mice (11). Moreover, marrow protected thymectomized irradiated mice grafted with allogeneic thymus tissue can also react against skin grafts and tumors of the same genetic makeup as that of the reconstituting thymus (12, 13), implying that the cells which manifest immune activity are derived from the bone marrow inoculum and attain competence after processing by the thymus. Similarly, *in vitro* contact between bone marrow cells, irradiated syngeneic spleen fragments, and allogeneic thymus tissue resulted in *graft-versus-host* activity of cells against tissue of the same genetic origin as the thymus used (14). Antibody formation after injection of bone marrow cells into irradiated thymectomized recipients bearing thymic tissue within Millipore diffusion chambers (15) also suggests that differentiation of bone marrow precursor cells occurs

under the influence of a noncellular thymic agent. Indeed, a humoral component of thymic tissue which can restore competence in immunologically deficient mice has been described (16, 17). The response of neonatally thymectomized and thymectomized irradiated mice to allogeneic skin and tumor grafts and the ability of spleen cells from these mice to carry out a *graft-versus-host* reaction were partially restored by injection of a humoral factor of thymic origin, and the activation of lymphoid cells by such a thymic factor was demonstrated after a brief period of contact *in vitro* (16).

In the light of all this evidence it seemed plausible that such a noncellular component of thymic tissue might be responsible for one step in a process by which bone marrow cells could acquire immunocompetence. In the course of the present experiments, performed to test this hypothesis, it became apparent that an extractable thymic factor does in fact influence the development of mouse bone marrow cells towards immunological competence, but attainment of capacity to induce a *graft-versus-host* response also seems to depend upon additional changes in these cells which occur within the peripheral lymphoid tissue. The evidence leading to this conclusion is presented here.

Materials and Methods

Experimental Animals.—C57BL/6 mice originally obtained from Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, and bred at the Weizmann Institute by sibling mating and (C3H/eb × C57BL/6)F₁ mice were used in these experiments. The animals were kept at 22°–26°C, weaned at 6 wk of age, and fed Purina Laboratory Chow pellets and tap water *ad libitum*. Neonatal thymectomy was performed within 24 hr after birth by a modification of Miller's technique (18), and adult thymectomy was performed at 8 wk of age by a technique similar to that described by Kaplan (19). Any animal found to contain a remnant of thymic tissue upon visual or histological examination was discarded from the experiment. Irradiation consisted of a single dose of 850 R whole body irradiation from a General Electric Maximar III 250 X-ray machine, 215 kvp, 15 ma, 40 R/min, using filters of 0.5 mm Cu and 1 mm Al at a target distance of 50 cm in a field of 20 × 20 cm.

Cell Suspensions.—Bone marrow cells were obtained from the tibia and femora of young adult mice. A 24 gauge needle mounted on a syringe containing cold culture medium (Eagle's basal medium, supplemented with 10% Difco horse serum [Difco Laboratories, Detroit, Mich.] and 5% chick embryo extract) was inserted into one end of the bone and the marrow was discharged by forcing the solution through the marrow cavity. Thymuses or spleens were removed from mice and dispersed by pressure through a stainless steel mesh into cold culture medium. After passage of bone marrow, thymus, or spleen cells through 27-gauge needles, aliquots of cells were stained with Turk's solution and nucleated cells were counted in a hemacytometer.

Extracts.—Extracts of thymus, lymph node, and spleen from calf and thymus extract from C57BL/6 mice were prepared as described previously (20, 21). Crude preparations (100,000 g supernatant after 5 hr centrifugation of calf extracts or 35,000 g supernatant after ½ hr centrifugation of mouse extract) were used in these experiments. Extracts containing 10 mg/ml protein were diluted 1:50 in culture medium and suspensions of bone marrow cells were incubated in these solutions for 1 hr in a 37°C shaking water bath.

Test of Immunocompetence.—Immunocompetence of each cell population under test was evaluated by the ability of these cell suspensions to induce a *graft-versus-host* response accord-

ing to the in vitro method developed by Auerbach and Globerson (22). The presence of competent cells within a cell suspension assayed was indicated by the enlargement of (C3H/eb × C57BL/6)F₁ newborn spleen fragments 4 days after challenge. As described previously (20), a culture was considered reactive when the index of splenomegaly obtained was 1.2 or more.

Experimental Design.—Donor cells were treated according to one of the following procedures (illustrated in Fig. 1): (a) 1 million or 3×10^6 C57BL/6 bone marrow cells were tested for immunocompetence either directly after removal from the donor mice or after 1 hr of incubation in thymus extract.

(b) C57BL/6 or (C3H/eb × C57BL/6)F₁ bone marrow cells were incubated for 1 hr in thymus extract and the cells concentrated by centrifugation without further washing were injected into one of the following types of young adult recipient animals:

- B₁ neonatally thymectomized C57BL/6 mice which received 20×10^6 or 50×10^6 nucleated bone marrow cells intraperitoneally
- B₂ adult thymectomized irradiated C57BL/6 mice which received 50×10^6 nucleated bone marrow cells intravenously
- B₃ intact (C3H/eb × C57BL/6)F₁ mice which received 100×10^6 nucleated bone marrow cells intravenously

Control C57BL/6 bone marrow cells were incubated in culture medium or in extracts of lymph node or spleen and injected into similar recipients. Control (C3H × C57BL/6)F₁ bone marrow cells were incubated in thymus extract and similarly injected. After 24 hr (B₁ and B₃) or 7–9 wk (B₂), 1×10^6 cells from the spleens of these recipient mice were tested in the graft-*versus*-host assay used to evaluate immunological competence.

(c) C57BL/6 bone marrow cells were incubated for 1 hr with thymus extract and 1×10^6 cells were then added in vitro to $2 \times 2 \times 1$ mm fragments of spleen tissue from irradiated (C3H × C57BL)F₁ mice. After 24 hr under culture conditions described previously (20), 1×10^6 cells in suspensions prepared from these cultures were tested for immunocompetence. Each test suspension thus containing a mixture of C57BL bone marrow cells and irradiated F₁ spleen cells was compared with a reference suspension containing F₁ bone marrow cells cultured similarly. Control C57BL/6 bone marrow cells were either incubated in culture medium and similarly added to fragments of irradiated F₁ spleen, or incubated in thymus extract and added to fragments of irradiated F₁ thymus, and then tested for graft-*versus*-host activity.

(d) C57BL/6 thymus cells were added in vitro to $2 \times 2 \times 1$ mm fragments of lymph node tissue from untreated or irradiated F₁ mice. After 24 hr under culture conditions described previously (20), 1×10^6 cells in suspensions prepared from these mixed cultures were tested for immunocompetence. Control C57BL thymus cells were tested directly in the graft-*versus*-host assay, or assayed with the addition of 1×10^6 dissociated F₁ lymph node cells, or cultured with fragments of F₁ thymus tissue and then tested for immunocompetence.

RESULTS

The working hypothesis tested in the present experiments proposed that cells located in the bone marrow can acquire immunological competence by a process that involves a noncellular component of the thymus. As a criterion of immunological competence, the ability of cells to induce a graft-*versus*-host response was tested according to the in vitro method developed by Auerbach and Globerson (22). The first experiment was thus performed to evaluate the activity of untreated bone marrow cells in this graft-*versus*-host assay. Spleen explants from newborn (C3H × C57BL)F₁ hybrid mice were challenged by 1×10^6 or 3×10^6 bone marrow cells from adult C57BL donors. Splenomegaly

of the explants under challenge in comparison to the paired reference explants did not exceed an index of 1.2, which we consider as the minimal degree of reactivity which indicates the presence of competent graft cells (20). Thus, as shown in Table I, immunologically competent cells were not detected within the bone marrow populations by this assay. Suspensions of bone marrow cells were then tested after incubation in calf thymus extract, since this treatment was previously found adequate for immune activation of spleen cells obtained from thymectomized mice (20). However, as can be seen in Table I, this treatment with thymus extract was insufficient to endow bone marrow cells with immune competence as indicated by the failure to induce significant splenomegaly. On the other hand, in experiments cited previously, immunocompetent cells derived from the bone marrow and apparently processed by the thymus

TABLE I
*Graft-versus-Host Assay of C57BL Bone Marrow Cells Incubated in Calf Thymus Extract**

Number of bone marrow cells tested	Extract	Incidence of reactive cultures‡		
1×10^6	Thymus	0/5	0/5	1/10
1×10^6	—	0/4	0/5	0/4
3×10^6	Thymus	0/5	1/5	0/10
3×10^6	—	0/4	0/4	

* Bone marrow cells were incubated for 1 hr in calf thymus extract at a concentration of 0.02 g protein/100 ml culture medium.

‡ Number of cultures of (C3H \times C57BL) F_1 spleen explants with spleen index ≥ 1.2 per total number of cultures tested.

have been recovered from the thoracic duct and other peripheral lymphoid organs. It thus seemed plausible that bone marrow cells achieve full immune reactivity only after reaching the peripheral lymphoid tissues. We then proceeded to test cells recovered from the spleen of immunologically impaired animals injected with bone marrow cells previously exposed to thymus extract. Suspensions of bone marrow cells taken from adult intact C57BL mice were incubated together with calf thymus extract for 1 hr and 20×10^6 or 50×10^6 of these cells, or aliquots of cells incubated in spleen extract or in culture medium only, were then injected intraperitoneally into syngeneic young adult mice thymectomized neonatally (Fig. 1, B_1). 24 hr after administration of bone marrow suspensions, 1×10^6 spleen cells of these thymectomized mice were tested in the graft-versus-host assay. As seen in Table II, a clear indication of immunological activity was apparent when spleen cell suspensions assayed were prepared from the mice injected with bone marrow cells previously exposed to thymus extract, whereas spleen cell suspensions prepared from control animals

were without detectable immune reactivity. To counter the objection that the role of bone marrow cells in this process was to carry a residual amount of thymus extract which might have been sufficient to activate spleen cells of the thymectomized recipients, spleen cells of thymectomized C57BL mice were assayed after injection of F₁ bone marrow cells similarly incubated in thymus extract. No graft-*versus*-host activity was apparent, however, as can be seen in Table II.

In an alternative procedure aimed to increase the proportion of donor cells in the population under test, bone marrow cells incubated in an extract of calf

TABLE II
Graft-versus-Host Response Induced by Spleen Cells from Neonatally Thymectomized C57BL/6 Mice Injected with Bone Marrow Cells Preincubated in Calf Thymus Extract†*

Extract tested	Bone marrow cell donor	Number of bone marrow cells injected	Incidence of reactive cultures‡				
Thymus	C57BL	50 × 10 ⁶	5/5	4/7	5/8	2/6	4/5
—	C57BL	50 × 10 ⁶	1/7	1/8	0/5	1/6	0/5
Thymus	(C3H × C57BL) _F ₁	50 × 10 ⁶	0/5	0/5	1/5		
Thymus	C57BL	20 × 10 ⁶	3/5	2/5	3/4		
Spleen	C57BL	20 × 10 ⁶	0/4	0/5	1/5		

* 24 hr after bone marrow cell injection, 1 × 10⁶ spleen cells of the thymectomized recipients were assayed.

† Bone marrow cells were incubated for 1 hr at 37°C in extracts at a concentration of 0.02 g protein/100 ml culture medium.

‡ Number of cultures of (C3H × C57BL)_F₁ spleen explants with a spleen index ≥ 1.2 per total number of cultures tested.

thymus were injected into adult thymectomized irradiated recipients (Fig 1, B₂). C57BL mice thymectomized at 2 months of age were irradiated 1 wk later with 850 R and injected intravenously within 2 hr with 50 × 10⁶ nucleated syngeneic bone marrow cells or bone marrow cells previously incubated in calf thymus extract. As shown in Fig. 2, 7–9 wk later the spleen cells from those mice that were repopulated by bone marrow cells which had been exposed to thymus extract evidenced the capacity to induce a graft-*versus*-host reaction, whereas this capacity was not apparent in control mice repopulated by untreated bone marrow cells. Thus, immunologically competent cells were again demonstrated within the spleen when immunologically defective mice were inoculated with thymus-treated bone marrow cells. These competent cells could still be detected several weeks after injection.

The assumption that the reactive cells in these spleen cell preparations were of donor bone marrow origin was submitted to further test in the following ex-

periments (Fig. 1, B_3). C57BL bone marrow cells were incubated for 1 hr in calf thymus extract and 100×10^6 of these cells were injected intravenously into intact (C3H \times C57BL) F_1 recipients. 1×10^6 spleen cells in suspensions obtained from these recipients 24 hr later were then tested in the graft-*versus*-host assay against tissue of the same (C3H \times C57BL) F_1 origin. Cells competent to initiate a graft-*versus*-host reaction were found within the spleen of each of those intermediate (C3H \times C57BL) F_1 mice injected with C57BL bone marrow

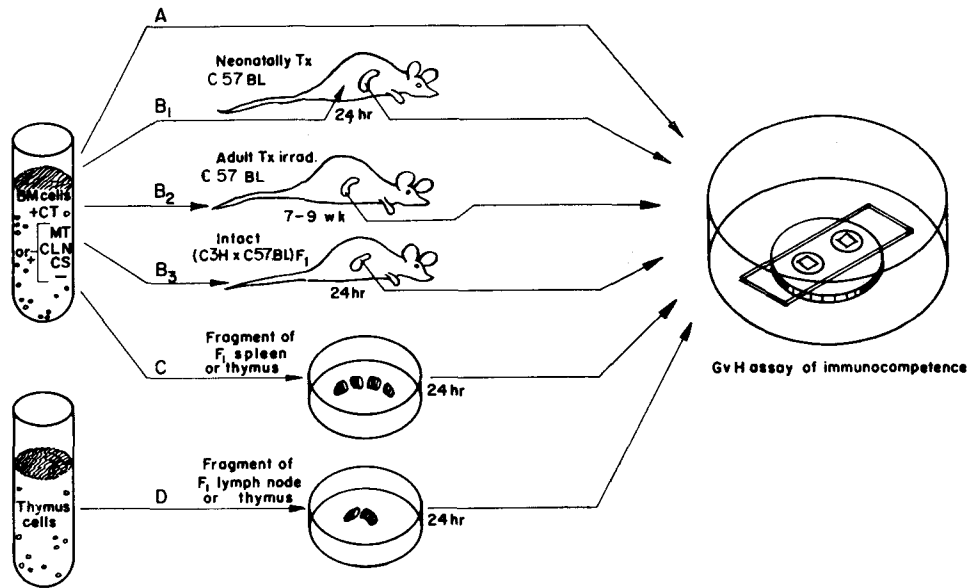


FIG. 1. Scheme of experimental procedures. Suspensions of bone marrow cells (*BM*) incubated in extract of calf thymus (*CT*), mouse thymus (*MT*), calf lymph node (*CLN*), calf spleen (*CS*), or without extract, and thymus cell suspensions were transferred in vivo or in vitro and tested for competence as illustrated and described in the text.

cells previously exposed to thymus extract, while mice injected with cells of the same parental bone marrow incubated in culture medium only did not manifest reactivity (Table III and Fig. 3). It was concluded that cells originating in the bone marrow inoculum had indeed attained the capacity to initiate a graft-*versus*-host response. Moreover, F_1 bone marrow cells incubated in thymus extract and injected into F_1 intermediates were inactive when tested against F_1 tissue as can be seen in Table III. This control again points to the bone marrow inoculum as the source of the active cells. All of these experiments were performed with thymic extracts of bovine origin. When extract prepared from C57BL mouse thymus was tested, activation of syngeneic bone marrow cells was also observed after these cells were recovered from F_1 spleens as shown in

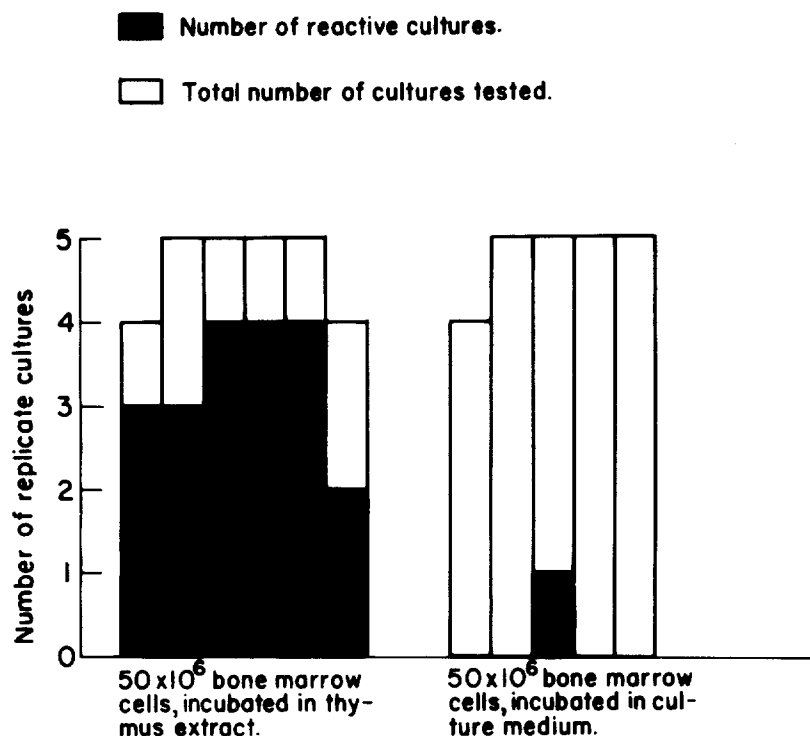


FIG. 2. Graft-versus-host response induced by spleen cells from adult thymectomized C57BL mice 7-9 wk after lethal irradiation and injection of bone marrow cells preincubated in calf thymus extract. Control mice were injected with bone marrow cells incubated in culture medium alone. (C3H × C57BL)_F₁ spleen explants challenged by spleen cells from these recipients were considered reactive when the index of splenomegaly was ≥ 1.2 .

TABLE III

Graft-versus-Host Response Induced by Bone Marrow Cells Incubated in Thymus Extract, Injected into (C3H × C57BL)_F₁ Mice, and Recovered from the Spleens† of These Recipients*

Bone marrow cell donor	Incidence of reactive cultures‡									
	3/5	4/5	3/5	3/5	4/5	3/5	4/5	4/7	6/6	
C57BL										
(C3H × C57BL) _F ₁			0/5	1/5	0/5	1/6				

* 100×10^6 bone marrow cells were injected i.v. after 1 hr incubation in calf thymus extract at a concentration of 0.02 g protein/100 ml culture medium.

† 24 hr after bone marrow cell injection, 1×10^6 spleen cells of the (C3H × C57BL)_F₁ recipients were assayed.

‡ Number of cultures of (C3H × C57BL)_F₁ spleen explants with spleen index ≥ 1.2 per total number of cultures tested.

Fig. 3. Incubation of bone marrow cells in extracts of calf lymph node did not result in subsequent activity (Fig. 3), thus implicating a factor of specifically thymic origin in the process conferring competence upon the bone marrow cells.

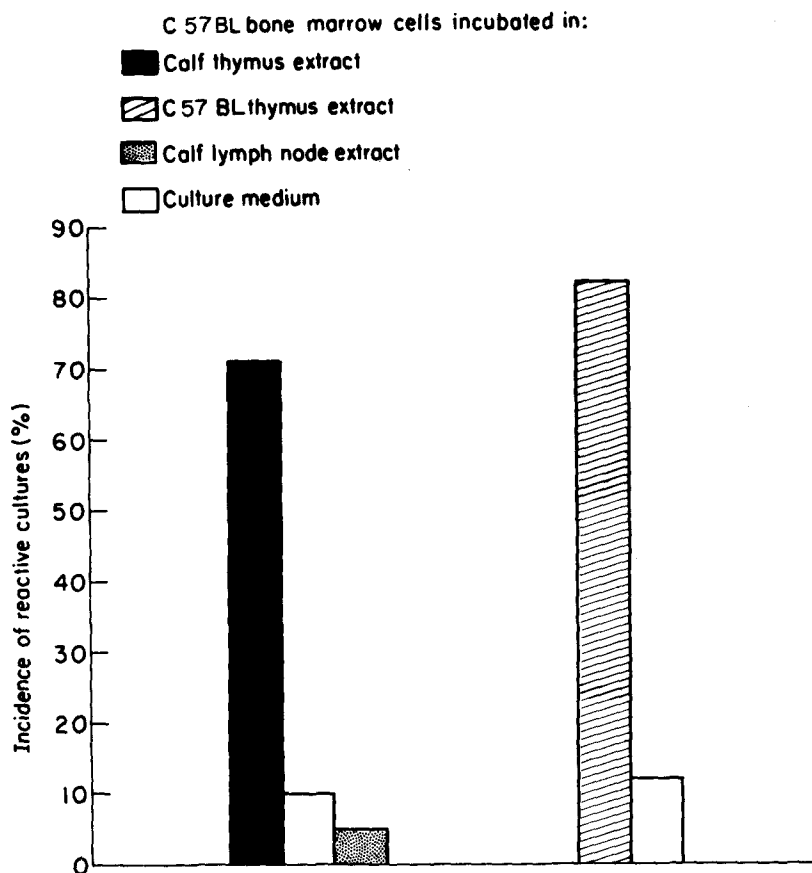


FIG. 3. Graft-versus-host response induced by bone marrow cells from C57BL mice after incubation in extract of calf thymus or syngeneic mouse thymus and injection into (C3H \times C57BL) F_1 mice. Control bone marrow cells incubated in calf lymph node extract or in culture medium alone were similarly injected. Cells recovered from the spleens of the recipient mice were assayed against spleen explants of the same (C3H \times C57BL) F_1 strain. A spleen index ≥ 1.2 was considered evidence of reactivity.

From the results of all of these experiments it appears that bone marrow cells can differentiate to immunologically competent cells under the influence of an extractable component of thymic tissue, acting in collaboration with other mediators of differentiation located in the peripheral lymphoid tissues.

It then seemed possible that cells which are already in contact with the humoral factor present within the thymic environment might similarly manifest full immune competence after migration to lymph nodes or spleen. To test this possibility, suspensions of C57BL thymus cells in numbers insufficient to induce a graft-*versus*-host response were added in vitro to mesenteric lymph node fragments from (C3H \times C57BL) F_1 mice, and 24 hr later the immunocompetence of cell suspensions prepared from these cultures was tested by in vitro assay of graft-*versus*-host activity against F_1 tissue (Fig. 1,D). The results sum-

TABLE IV
Graft-versus-Host Response Induced by C57BL Thymus Cells Exposed to (C3H \times C57BL) F_1 Lymph Node Tissue and Tested Against (C3H \times C57BL) F_1 Spleen Explants

Cells tested	Incidence of reactive cultures*				
C57BL thymus cells‡	0/5	1/5	2/5	0/5	0/4
C57BL thymus cells cultured with F_1 lymph node tissue fragments§	4/7	6/9	3/5	4/5	3/4
C57BL thymus cells cultured with irradiated F_1 lymph node tissue fragments		5/7		5/8	
C57BL thymus cells cultured with F_1 thymus tissue fragments§		0/7	0/10		0/5
C57BL thymus cells assayed with addition of F_1 lymph node cells¶		0/5		2/15	

* Number of cultures with spleen index ≥ 1.2 per total number of cultures tested.

‡ 1×10^6 thymus cells were assayed.

§ After 24 hr of contact in vitro between 1×10^6 thymus cells and tissue fragments, 1×10^6 cells obtained from these mixed cultures were assayed.

|| After 24 hr of contact in vitro between 1×10^6 thymus cells and tissue fragments from mice preirradiated with 850 R, 0.5×10^6 cells obtained from these mixed cultures were assayed.

¶ 1×10^6 thymus cells were assayed together with 1×10^6 F_1 lymph node cells.

marized in Table IV indicate that suspensions prepared from C57BL thymus cells cultured together with F_1 lymph node fragments did indeed manifest the presence of competent cells of thymic genotype since a positive graft-*versus*-host reaction was observed. The same results were observed when thymic cells cultured with lymph node fragments from lethally irradiated F_1 mice were tested for graft-*versus*-host activity. When suspensions of C57BL thymus cells were cultured with fragments of F_1 thymus instead of lymph nodes no activity was evident. Also, suspensions of 1×10^6 thymus cells tested with addition of 1×10^6 dissociated F_1 lymph node cells were without activity. Thus, one of the steps in the differentiation of these cells to immunocompetence appears to involve a process which occurs outside the thymus, within the environment of the peripheral lymphoid organs.

It was then considered necessary to reproduce in vitro the whole process of immunologic maturation starting with inactive bone marrow cells in order to confirm the validity of the conclusions derived from experiments involving pas-

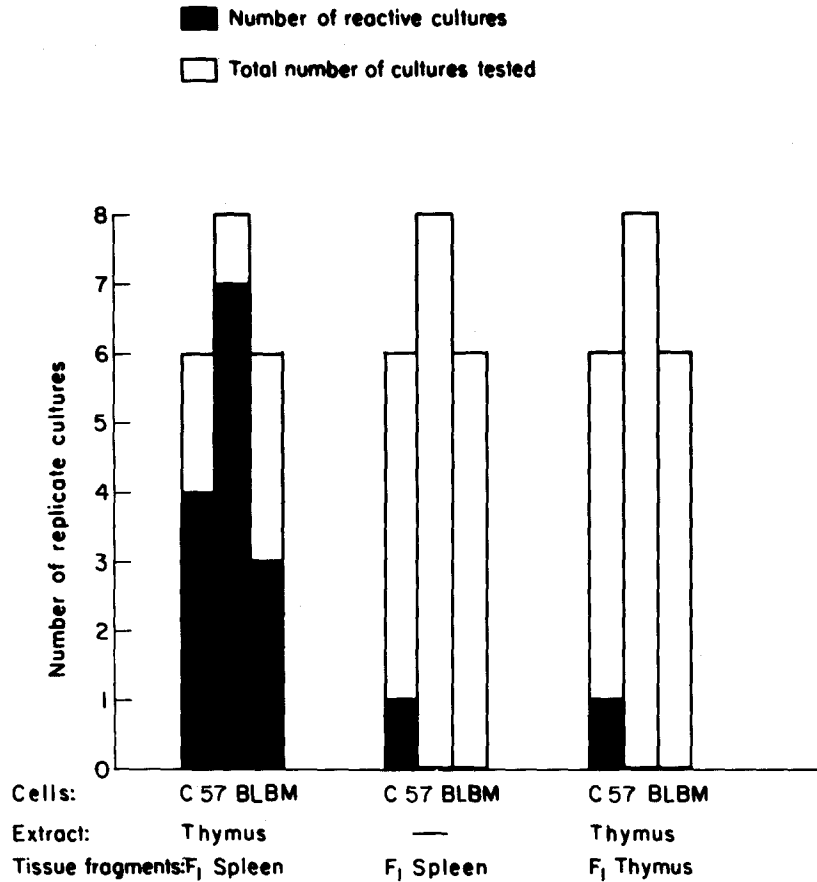


FIG. 4. Graft-versus-host response induced by C57BL/6 bone marrow cells after incubation with thymus extract and in vitro contact with fragments of spleen tissue from lethally irradiated (C3H \times C57BL)F₁ mice. Control C57BL bone marrow cells were incubated in culture medium alone and added to F₁ spleen fragments, or incubated in thymus extract and added to F₁ thymus fragments. (C3H \times C57BL)F₁ spleen explants challenged with cells from these mixed cultures were considered reactive when the spleen index was ≥ 1.2 .

sage of cells through intermediate mice. For this purpose, cells from C57BL bone marrow were incubated for 1 hr in calf thymus extract, then added to spleen explants from lethally irradiated F₁ mice, and 24 hr later tested against spleen tissue of the same F₁ origin (Fig. 1,C). In parallel control cultures, cells

from the same bone marrow suspension incubated in culture medium were added to similar spleen fragments, and in another control, bone marrow cells incubated with thymus extract were added to thymus fragments from the same irradiated F₁ mice. As can be seen in Fig. 4, by in vitro contact with thymus extract and with peripheral lymphoid tissue, bone marrow cells acquired the capacity to induce a graft-*versus*-host reaction. These components would thus appear to be sufficient as well as necessary for the immunodifferentiation process leading to graft-*versus*-host activity.

When thymic fragments were used instead of spleen fragments, activity of bone marrow suspensions was not observed (Fig. 4). Thus, the maturation process seems to require the environment of the peripheral lymphoid tissue for a function which is still present in spleen fragments from mice exposed to 850 R whole body irradiation.

DISCUSSION

Although participation of the thymus is known to be essential for lymphoid cell activity in immune responses of the cell-mediated type, the nature of the relation between the thymus and the cells carrying out such responses remains to be clarified. A noncellular component of thymic tissue can mediate some aspects of this function as shown by restoration of the homograft response in neonatally thymectomized or adult thymectomized irradiated mice after repeated injections of cell-free thymic preparations (23). An interaction at the cellular level seems to underlie the repair observed in these thymectomized mice, since cells from similar thymectomized donors were found to attain the capacity to initiate a graft-*versus*-host response after a brief in vitro incubation with thymic extracts (20). Thus, it appeared that direct contact between the thymus factor and immunologically incompetent cells is adequate to trigger that step on the pathway towards immunocompetence which is thymus dependent. In view of the evidence indicating thymic involvement in immunodifferentiation of precursor cells from the bone marrow to competent cells of the lymphoid system, it seemed likely that this noncellular thymic factor should activate the incompetent cells which arise in the bone marrow.

In the series of experiments described here, nonreactive cells from mouse bone marrow did indeed acquire the capacity to react in an in vitro graft-*versus*-host assay after incubation with extracts of thymic origin. However, contact between the extract-treated cells and peripheral lymphoid tissue also seemed to be essential in order for these cells to exhibit reactivity. Specifically, when bone marrow cells were injected into mice incapable of inducing a response in the graft-*versus*-host assay (as a result of neonatal thymectomy or adult thymectomy plus irradiation, or because of genetic similarity with the tissue used for challenge in the assay) competent cells were recovered from the spleens of these recipient mice if the bone marrow inoculum had been previously incubated in thymus extract of xenogenic or syngeneic source. Competent cells were not

detected in such recipients when bone marrow cells had been incubated in xenogeneic spleen or lymph node extract, however, indicating that a factor present in the thymus is required to trigger immunodifferentiation of bone marrow cells. The cells activated by the thymus factor in this procedure were contributed by the bone marrow inoculum, since immune reactivity depended upon the genetic makeup of the bone marrow cells rather than that of the intermediate recipients. It thus seems that the potential to initiate a response against foreign tissue resided in the bone marrow cells in a latent form until activated by the factors controlling further differentiation.

The interaction between a thymic factor and bone marrow cells appears to be only one step in the process leading to immunocompetence, since contact between marrow cells and thymus extract was not sufficient per se to enable graft-*versus*-host initiation by these cells. An additional process appeared to occur within the environment provided by the peripheral lymphoid organs as indicated by the attainment of competence by bone marrow cells preincubated in thymus extract and exposed to nonresponsive spleen tissue in vitro as well as in vivo. This process seems to depend upon some component found within peripheral lymphoid tissues, since extract-treated bone marrow cells failed to exhibit immune reactivity after in vitro contact with thymus tissue. This interpretation is supported by the demonstration that suspensions of thymic cells also manifested increased reactivity after in vitro association with genetically unresponsive lymph node tissue.

Although further investigation is required to determine whether the cells which were activated by a humoral thymic factor in these experiments were in fact stem cells which had never left the bone marrow or thymus-derived cells which may also be found within the bone marrow population (24), we suggest that precursor cells from the bone marrow can acquire immunocompetence vis-a-vis graft-*versus*-host responses by a progressive differentiation process. The present results indicate that one stage is under the control of an agent that can be extracted from the thymus and a complementary step depends upon some unidentified components located within the peripheral lymphoid tissue environment. According to this concept we envisage successive steps of lymphocyte differentiation occurring in separate compartments of the lymphoid system. Cells already processed by the thymus could thus attain immunocompetence upon migration to the peripheral lymphoid organs. Incidentally, the immune reactivity shown by thymus cells after in vitro contact with lymph node tissue from mice both lethally irradiated and genetically unable to react in the assay suggests that the graft-*versus*-host response was initiated by the thymus-processed cells alone. Thus, instead of synergistic activity of two types of lymphoid cells reported in some investigations (25, 26), our results suggest that one class of cells may acquire immune reactivity by progressive development.

In an early work the suggestion was made that the thymus, bone marrow,

and spleen participate together in an inductive process which leads to the development of immunologically competent cells (27). Also, conferment of graft-*versus*-host reactivity upon bone marrow cells by another thymic preparation has been recently reported (17). While those results also argue in favor of an interaction between a thymic humoral factor and bone marrow cells, our experiments indicate the need for additional steps in the process leading to immunological maturity. Further experiments will determine the relevance of these observations to the natural pathway followed by lymphocyte precursors in order to reach immunological competence.

SUMMARY

The hypothesis that cells located in mouse bone marrow can acquire immunological competence by a process that involves interaction with a noncellular component of the thymus was tested using an *in vitro* assay of graft-*versus*-host reactivity as a criterion of cell competence. When suspensions of C57BL bone marrow cells were incubated in thymus extract and injected into mice incapable of inducing a response in the graft-*versus*-host assay as a result of neonatal thymectomy, or adult thymectomy plus irradiation, or because of genetic similarity with the (C3H \times C57BL) F_1 tissue used for challenge in the assay, competent cells were recovered from the spleens of the injected mice. The reactive cells were shown to be of bone marrow origin since immune reactivity was related to the genetic makeup of the bone marrow cells rather than that of the intermediate recipients. A thymic factor was involved in the process leading to immune reactivity by these cells, as bone marrow cells incubated in xenogeneic or syngeneic thymic extracts induced a graft-*versus*-host response after passage through nonresponsive mice, whereas incubation of bone marrow cells in xenogeneic lymph node or spleen extracts or in culture medium only did not lead to subsequent reactivity. Participation of peripheral lymphoid tissue seemed essential in this process since bone marrow cells tested directly after exposure to thymic extract failed to induce a graft-*versus*-host response. C57BL bone marrow cells exposed to thymus extract and cultured together with fragments of (C3H \times C57BL) F_1 spleen tissue *in vitro* were competent to induce a graft-*versus*-host response; thus, these components would seem to be sufficient as well as necessary for the immunodifferentiation process leading to graft-*versus*-host activity.

It is concluded that one step in the process by which bone marrow cells acquire competence vis-a-vis the graft-*versus*-host response depends upon a thymic agent that is noncellular and extractable, and that another stage in this process is under the influence of components found within the peripheral lymphoid tissue environment. It is suggested that differentiation of precursor cells to competence could occur by progressive development of the cells in separate compartments of the lymphoid system.

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