IMMUNOCOMPETENCE OF SPLEEN CELLS FROM NEONATALLY THYMECTOMIZED MICE CONFERRED IN VITRO BY A SYNGENEIC THYMUS EXTRACT

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Contributions of the mammalian thymus to the immunological integrity of an animal begin during early life, when it is essential for development of the lymphoid system and full immunocompetence (1–3), and continue in adult life as shown under conditions requiring replenishment of the population of immunologically active cells (4–8). At least part of these functions appear to be mediated by a humoral mechanism since most of the pathological effects of neonatal thymectomy can be prevented by a noncellular component of the thymus. Partial recovery of the skin homograft response and of primary antibody response against sheep erythrocytes was observed in mice implanted with syngeneic or allogeneic thymus tissue in cell-impermeable diffusion chambers. Such mice showed neither depletion of lymphocytes of peripheral blood and lymphoid organs, nor characteristics of the wasting syndrome which follows thymectomy of newborn animals (9–12). Indications of restored immunological structure and function were also obtained in similar experiments in rats, hamsters, and rabbits (13–15). The noncellular nature of this thymic agent has been confirmed by studies in mice on the reparative effects of a cell-free component prepared from thymus of syngeneic or xenogeneic species. A humoral factor capable of increasing the lymphocyte population in the spleen and peripheral blood of intact and thymectomized mice has been obtained from the thymuses of mice, sheep, calves, and rabbits (16–19). Wasting disease of neonatally thymectomized mice was prevented by administration of such extracts (19). Repeated injections of calf thymus preparations led to partial restoration of the ability to reject allogeneic skin grafts and tumors (20, 21) and to form antibodies in a primary response against sheep erythrocytes (22). Repeated injections of thymus extract from calves (20, 21) or extract of syngeneic origin (23) also restored the capacity of spleen cells of neonatally thymectomized mice to induce a graft-versus-host reaction, which indicates that immunocompetence of the cells initiating this response depends upon a thymic humoral factor.

The nature of the thymus-dependent process or processes underlying these

different immune reactions is still incompletely understood. We approached this question by considering the action of the thymus humoral factor at the cellular level and investigating its role in the attainment of immunocompetence by the cells which mediate a cell-bound reaction. An in vitro method of restoration seemed most appropriate to clarify the nature of the interaction between the humoral agent of thymic function and the ultimate immunologically active cells. The present experiments were therefore designed to test the possibility of a direct effect upon dissociated spleen cells from neonatally thymectomized mice by a cell-free component extracted from syngeneic thymus.

Materials and Methods

Mice.—C57B1/6 and (C3H/eb × C57B1/6)F_1 hybrids were used in these experiments. C57B1/6 and C3H/eb mice were originally obtained from Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, and were bred at the Weizmann Institute of Science by sibling mating. The animals were kept at 22°–26°C, fed Purina Laboratory Chow pellets and tap water *ad libitum*, and weaned at 6 wk of age. Thymectomy was performed within 24 hr after birth by a modification of Miller's technique (24). Any animal found to contain a thymic remnant was discarded from the experiment.

Preparation of Spleen Cell Suspensions.—Spleens were removed aseptically from 6–10 wk old thymectomized C57B1/6 male or female mice and dispersed by pressure through a stainless steel mesh into organ culture medium (described below) or Tyrode's solution. The cells were further dissociated by means of a syringe with 27 gauge needle. Aliquots of cells were stained with Türk's solution and nucleated cells were counted in a hemacytometer. Exclusion of 0.05% trypan blue solution was used as a measure of viability. Spleen cell suspensions were prepared in a similar manner from intact C57B1/6 mice or (C3H/eb × C57B1/6)F_1 mice either intact or thymectomized, aged 6 wk or more.

Preparation of Extracts.—Extracts were prepared from the thymuses of C57B1/6 female mice of approximately 3 months of age. For each preparation, 20 thymuses were removed aseptically and homogenized in 3 ml of cold 0.1 N sodium–phosphate buffer, pH 7.4 in a conical glass tissue grinder. The homogenate was centrifuged at 35,000 *g* for 30 min. After straining the supernatant through gauze, we determined protein concentration by the biuret reaction and diluted the solution to contain 10 mg protein/ml. To exclude cells and to sterilize, we passed extracts through Millipore filters (Millipore Filter Corp., Bedford, Mass.) of pore size 0.45 μ. Extracts of spleen or mesenteric lymph node were prepared from the same mice by a similar procedure and adjusted to the same protein concentration. Prior to use, the extracts were stored at −10°C and diluted to the desired concentration in either culture medium or Tyrode's solution.

Test of Immunocompetence.—Immunocompetence of dissociated spleen cells was evaluated by means of an in vitro assay of graft-versus-host reactivity. The relative enlargement of a (C3H/eb × C57B1/6)F_1 spleen explant challenged by parental cells as compared with a paired explant exposed to cells of syngeneic (C3H/eb × C57B1/6)F_1 origin reflected response to challenge by immunocompetent cells. In accordance with the method developed by Auerbach and Globerson (25), cultures of paired spleen fragments approximately 1.0 × 1.0 × 1.5 mm in size were prepared from (C3H/eb × C57B1/6)F_1 mice less than 24 hr of age. Cultures of paired spleen explants were maintained in Millipore filter wells overlaying 1 ml of medium. The culture medium consisted of Eagle's basal medium supplemented with 10% horse serum (Difco No. 5357, Difco Laboratories, Detroit, Mich.), 5% chick embryo extract (prepared from 9-day embryos in an equal volume of Tyrode's solution), penicillin (100 u/ml), and streptomycin

(100 μg/ml). Two spleen fragments of equal dimensions were explanted in each culture dish and an inoculum of cells in 0.005 ml volume was added to each well by an orally controlled micropipette. Cultures were maintained in a 37°C incubator in a water-saturated atmosphere of 90% oxygen and 10% CO_2. These cultures were then checked after 1 day to ascertain that spleen explants of each pair were matched in size by measurement of the longest perpendicular diameters with an ocular micrometer at 10 × magnification. Relative size of the cultures was determined 4 days after the addition of cells. The calculated area of each test spleen fragment divided by the corresponding area of its paired reference fragment provided a numerical index of splenomegaly. Cultures were considered reactive when the index of splenomegaly obtained was 1.2 or more.

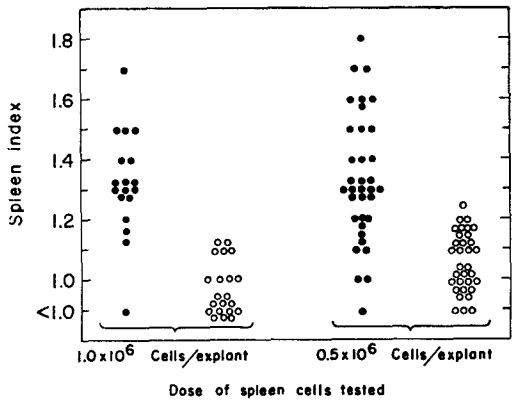
Exposure of Spleen Cells to Extract.—In experiments in which spleen cells from C57B1/6 thymectomized mice were exposed to extracts of thymus, spleen, or mesenteric lymph node during the graft-versus-host assay, 0.3 ml of each extract, prepared as described above, was diluted in 15 ml culture medium and used in place of the regular nutrient medium surrounding each pair of cultures.

In experiments in which the cells from C57B1/6 thymectomized mice were incubated with extracts prior to the graft-versus-host assay of immunological activity, 0.5 ml aliquots of dissociated spleen cells in either Tyrode's solution or culture medium were added to equal volumes of diluted extracts. Spleen cells from (C3H/eb × C57B1/6)F_1 controls received the same treatment. After incubation at 37°C the cells were washed three times in a volume of 5 ml Tyrode's solution or culture medium, centrifuged for 3 min at 1500 rpm, resuspended, counted, and added to newborn spleen explants for assay of immunocompetence. Incubation of 1 hr was carried out in a shaking water bath, whereas incubation over longer time intervals was without shaking in an atmosphere of 10% CO_2/90% air.

RESULTS

Lymphoid cells from immunocompetent mice injected into allogeneic newborn recipients initiate a graft-versus-host reaction manifested by a characteristic syndrome in the host which includes a marked enlargement of the spleen (26, 27). Splenomegaly can also be produced and measured in vitro by comparing the relative size of two matched spleen fragments: one challenged by nonsyngeneic lymphoid cells and the other exposed to lymphoid cells of syngeneic origin (25). The failure of spleen cells from neonatally thymectomized mice to evoke a graft-versus-host reaction when injected into genetically appropriate recipients has been shown in various experimental systems (28–30). Consequently, our first experiments were carried out to determine whether the difference in degree of the immunocompetence of spleen cells from neonatally thymectomized mice vis-a-vis cells from intact animals could also be detected by means of the in vitro assay of graft-versus-host reactivity. For this purpose spleen cells from young adult C57Bl/6 mice were tested for their ability to initiate enlargement of spleen explants from newborn (C3H/eb × C57Bl/6)F_1 hybrids in vitro as compared with equivalent fragments challenged by syngeneic F_1 spleen cells. Spleen cell inocula were prepared from intact mice or neonatally thymectomized donors. As can be seen in Fig. 1, 1×10^6 or 0.5×10^6 spleen cells from intact mice produced a response with a spleen index of 1.2 or more in almost every culture; thus, they were considered reactive. In comparison, the

same number of cells from thymectomized mice did not induce significant splenomegaly. As shown in Table I, the response evoked by cells from thymectomized donors occurred only sporadically, while enlargement of most of



1. Graft-versus-host response induced by spleen cells from intact or neonatally thymectomized C57B1/6 mice. Spleen explants from (C3H/eb × C57B1/6)F_1 newborn mice were challenged by dissociated spleen cells from intact (●) or neonatally thymectomized (○) donors. Each point represents one culture.

TABLE I

Graft-versus-Host Response Induced by Spleen Cells from Intact or Neonatally Thymectomized C57B1/6 Mice in (C3H/eb × C57B1/6)F_1 Newborn Spleen Explants

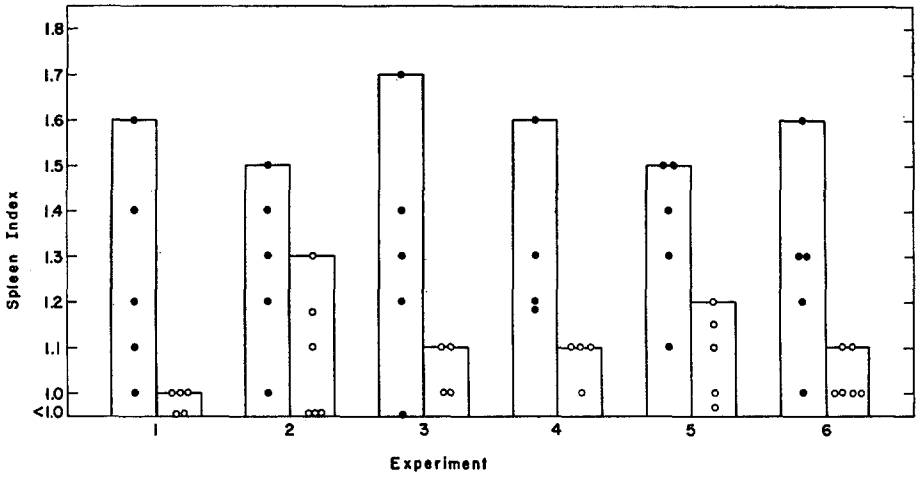
Donor of spleen cells	No. of cells tested/Explant	Incidence of reactive cultures*						
Intact	1.0×10^6	4/5	3/5	3/4	5/5			
Thymectomized	1.0×10^6	0/6	0/5	0/5	0/5			
Intact	0.5×10^6	3/4	5/6	4/6	3/4	4/5	4/5	4/5
Thymectomized	0.5×10^6	0/6	1/4	0/4	0/6	1/4	0/4	1/5
Intact	0.1×10^6	1/5	0/4	0/5	0/5	1/5		

* Number of cultures with a spleen index $\geq$ 1.2 per total number of cultures tested.

the replicate cultures challenged by cells from each intact mouse indicated immunocompetence of the donor cells. Since inocula of 0.1×10^6 cells from intact donors did not produce a significant augmentation of the spleen index, further experiments were performed with either 0.5×10^6 or 1×10^6 cells.

Once impairment of immunological activity of spleen cells from thymectomized mice was demonstrated by this in vitro assay, experiments were performed to investigate the possibility of direct reactivation of these incompetent cells by a humoral factor present in thymic extracts. Culture medium supple-

mented with syngeneic thymic extract (2 ml thymus extract containing 0.02 g protein/100 ml culture medium) was used in place of the regular culture medium surrounding the F_1 spleen fragments under challenge by 0.5×10^6 C57Bl/6 spleen cells from thymectomized donors. Cultures exposed to thymus extract were compared with cultures in regular medium. Dissociated spleen cells from thymectomized mice were thus exposed to thymic extract throughout the



2. Graft-versus-host response induced by spleen cells from neonatally thymectomized C57B1/6 mice in the presence of syngeneic thymus extract. Dissociated spleen cells from thymectomized donors and (C3H/eb × C57B1/6)F_1 spleen explants were maintained throughout the course of the 4 day assay in culture medium (○) or culture medium supplemented with thymus extract (●). The spleen index of the four to six replicate cultures tested in six individual experiments is shown.

course of the 4 day test period. As shown in Fig. 2, the capacity of spleen cells from thymectomized mice to induce splenomegaly was manifested when the thymic extract was present during the assay, while cells from thymectomized animals maintained in regular culture medium failed to induce significant splenomegaly. Possible variations in the level of immune impairment in individual thymectomized mice could not affect these results since suspensions of spleen cells from each donor were divided between cultures maintained in regular medium and cultures supplemented with thymus extract. Intact (C3H/eb × C57Bl/6)F_1 mice provided the syngeneic spleen cells used in these experiments. To show that the observed splenomegaly was an expression of immune reactivity and not the mere proliferation of cells from a thymectomized donor treated with thymus extract, we used spleens of (C3H/eb × C57B1/6)F_1 thymectomized mice as a source of syngeneic cells in additional experiments. Also in this case, the results obtained were similar to those shown in Fig. 2.

The specificity of action of the thymus extract was investigated by comparing its effect to that of spleen and mesenteric lymph node extract. Spleen explants prepared as previously described were maintained either in regular culture medium or in medium supplemented with extract (2 ml extract containing 0.02 g protein/100 ml medium) prepared from syngeneic thymus, spleen, or mesenteric lymph node. These cultures were challenged by 0.5×10^6 spleen cells from thymectomized donors; in each experiment a single donor provided the test cells for the cultures maintained in all types of medium. Incidence of reactive cultures in which the size of the test spleen fragments at 4 days exceeded by an

TABLE II

Graft-versus-Host Response Induced by Spleen Cells from Neonatally Thymectomized C57B1/6 Mice in the Presence of Syngeneic Thymus, Spleen, or Mesenteric Lymph Node Extract

Extract tested*	Incidence of reactive cultures‡				% of cultures responding
Thymus	3/5	3/4	9/10	4/5	79.0
Spleen	0/4	0/4	1/5	1/6	10.5
Mesenteric lymph node	0/5	1/5	2/10		15.0
—	1/4	0/4	1/5	0/6	10.5

* Syngeneic extracts were tested at a concentration of 0.02 g protein/100 ml culture medium throughout the course of the 4 day assay.

‡ Number of cultures with a spleen index $\geq$ 1.2 per total number of cultures tested. Each column represents one experiment in which spleen cells from the same donor mouse were tested against newborn (C3H/eb $\times$ C57B1/6)F_1 spleen explants.

index of 1.2 or more the size of control fragments exposed to syngeneic F_1 cells is presented in Table II. As shown in the table, only the spleen cells exposed to thymus extract initiated splenomegaly (19/24 cultures). Cells exposed to spleen extract or to mesenteric lymph node extract did not show greater reactivity than untreated cells from the same thymectomized donors (2/19, 3/20, 2/19 respectively).

Since, as shown above, spleen cells from thymectomized mice attained immunological competence during exposure to thymus extract in the presence of F_1 spleen fragments, the next step was the incubation of thymic extract with dissociated spleen cells alone prior to antigenic stimulation. In preliminary tests, aliquots of spleen cells from thymectomized donors were incubated for various intervals of time (17, 8, 4, and 1 hr) in regular culture medium or medium containing thymus extract. After washing, samples of 0.5×10^6 viable cells were added to spleen explants to test their ability to induce a graft-versus-host reaction. Significant splenomegaly was induced by the cells pretreated with thymus extracts for the different time intervals, indicating that restoration could occur as a result of a direct effect of thymus extract on the lymphoid cells. 1 hr incubation was used for further experiments, since more than 98% of the incubated spleen cells were viable after this time interval. At longer time

intervals the number of viable cells was reduced. Comparison of the restorative effect exerted by thymus extract at different concentrations indicated that 1 ml extract containing 0.01 g protein/100 ml medium was adequate. Consequently, spleen cells from thymectomized mice were incubated for 1 hr in a shaking water bath at 37°C in 1 ml Tyrode's solution or culture medium containing 0.1 mg extract protein prepared from syngeneic thymus, spleen, or

TABLE III

*Graft-versus-Host Response Induced by Spleen Cells from Neonatally Thymectomized C57B1/6 Mice Preincubated for 1 Hour in Syngeneic Thymus, Spleen or Mesenteric Lymph Node Extract**

Extract tested	Incubation medium	No. of cells tested/ (C3H/eb × C57Bl/6)F_1 explant	Incidence of reactive cultures‡		
Thymus	Culture medium	0.5×10^6	2/5	4/5	
—			0/5	0/5	
Thymus	Tyrode's	0.5×10^6	3/5	4/6	2/5
Spleen			0/10		
Mesenteric lymph node			0/5	0/5	
—			1/5	1/4	0/4
Thymus	Tyrode's	1.0×10^6	4/7	4/9	
Spleen			0/4	1/7	
Mesenteric lymph node			1/5	0/7	
—			0/4	1/7	

* Syngeneic extracts were tested at a concentration of 0.01 g protein/100 ml culture medium or Tyrode's solution.

‡ Number of cultures with a spleen index $\geq$ 1.2 per total number of cultures tested.

mesenteric lymph node. The cells were subsequently washed 3 times in 5 ml of Tyrode's solution or in culture medium, and immunocompetence was evaluated by the assay of splenomegaly. As can be seen in Table III, cells incubated with thymus extract induced splenomegaly in 23 out of 42 cultures tested, while cells exposed to extracts of spleen or mesenteric lymph node as well as untreated cells did not induce a significant incidence of response (5/77). These results demonstrate that spleen cells from thymectomized mice can attain immunological competence as a result of direct interaction with syngeneic thymus extract and also that this competence may be conferred in the absence of a simultaneous antigenic stimulation.

DISCUSSION

In these experiments a noncellular component of thymic tissue has been shown to participate directly in the chain of events leading to the immunological reactivity of lymphoid cells. When dissociated spleen cells from neonatally

thymectomized C57Bl/6 mice were exposed to syngeneic thymic extract in vitro, they attained the capacity to initiate a graft-versus-host response. This immunocompetence was endowed specifically by an extract of thymic origin since similar preparations from spleen or mesenteric lymph node at equivalent protein concentrations had no detectable effect. Moreover, spleen cells from thymectomized mice incubated for only 1 hr with thymus extract showed definite immunological competence as indicated by induction of the graft-versus-host response. The rapidity of this restoration points to a possible activating effect of thymus factor on target cells already present, rather than to the establishment of new cell populations by proliferation. Both the nature of this activation and the identity of the particular lymphoid cells affected are still matters of conjecture. The thymus-dependent stage in the induction of a graft-versus-host response could involve the ability of these lymphoid cells to recognize foreign antigens, or to transform to blasts and proliferate, or to damage the host tissue. Thus, alteration of the cell membrane, triggering of cell division, or changes in the pattern of enzymatic processes are some possibilities for further analysis. In any case, the results of the present experiments indicate that a thymus humoral factor can confer immunocompetence without the necessity for metabolic degradation or activation by the intact animal, and that the anatomic integrity of tissue containing the target lymphoid cells is not essential in order for thymus humoral factor to fulfill this function. Furthermore, neither migration of cells from other lymphoid organs nor replenishment of stem cells from the bone marrow was necessary for attainment of immunocompetence.

The role of the thymus in the immunological system of an animal has been explained by two types of hypotheses which do not necessarily exclude each other: on one hand, thymus-derived cells acting in the peripheral lymphoid organs have been implicated in immune responses (31–33), while experiments of different design have demonstrated a humoral mechanism of thymus function (9, 10, 20, 34, 35). A thymic humoral factor might act in the peripheral lymphoid tissues; or natural contact between this factor and its target cells could occur within the thymic environment, resulting in thymus-derived cells with immunological competence conferred by a humoral factor. The experiments discussed here present further evidence that a humoral thymus factor participates in the establishment of immunocompetence of lymphoid cells. In light of the present results, the specific role of this thymus factor appears to involve a rapid and direct activation of its target cells.

SUMMARY

Impaired immunological competence of spleen cells from neonatally thymectomized C57Bl/6 young adult mice was apparent when these cells were tested in an in vitro graft-versus-host assay. Spleen cell inocula prepared from

thymectomized mice did not induce enlargement of (C3H/eb × C57Bl/6)F_1 newborn spleen explants, whereas the same number of cells from intact donors consistently initiated splenomegaly. Spleen enlargement was observed, however, when the explants were challenged by cells from thymectomized donors in the presence of syngeneic thymus extract, indicating that the spleen cells in suspension attained immunological competence under the influence of a noncellular component of the thymus. Immunocompetence was also evident when the cells from thymectomized donors were first incubated with thymus extract for 1 hr and subsequently tested for reactivity. Cells from the same thymectomized donor mice exposed in parallel to extracts from syngeneic spleen or mesenteric lymph node at an equivalent protein concentration did not initiate a graft-versus-host response.

These experiments demonstrate that immune reactivity in the graft-versus-host response involves activation of lymphoid cells by a humoral factor of the thymus acting directly upon these cells.

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BIBLIOGRAPHY

1. Miller, J. F. A. P. 1961. Immunological function of the thymus. *Lancet* **2:**748.
2. Good, R. A., A. P. Dalmasso, C. Martinez, O. K. Archer, J. C. Pierce, and B. W. Papermaster. 1962. The role of thymus in development of immunologic capacity in rabbits and mice. *J. Exp. Med.* **116:**773.
3. Jankovic, B. D., B. H. Waksman, and B. G. Arnason. 1962. Role of the thymus in immune reactions in rats I. The immunologic response to bovine serum albumin (antibody formation, Arthus reactivity and delayed hypersensitivity) in rats thymectomized or splenectomized at various times after birth. *J. Exp. Med.* **116:**159.
4. Miller, J. F. A. P. 1962. Immunological significance of the thymus of the adult mouse. *Nature (London).* **195:**1318.
5. Feldman, M., and A. Globerson. 1964. The role of the thymus in restoring immunological reactivity and lymphoid cell differentiation in X-irradiated adult mice. *Ann. N.Y. Acad. Sci.* **120:**182.
6. Metcalf, D. 1965. Delayed effect of thymectomy in adult life on immunological competence. *Nature (London).* **208:**1336.
7. Taylor, R. B. 1965. Decay of immunological responsiveness after thymectomy in adult life. *Nature (London).* **208:**1334.
8. Linker-Israeli, M., and N. Trainin. 1968. Influence of adult thymectomy on growth of transplanted tumors in mice. *J. Nat. Cancer Inst.* **41:**411.
9. Levey, R. H., N. Trainin, and L. W. Law. 1963. Evidence for function of thymic tissue in diffusion chambers implanted in neonatally thymectomized mice. Preliminary report. *J. Nat. Cancer Inst.* **31:**199.
10. Osoba, D., and J. F. A. P. Miller. 1963. Evidence for a humoral thymus factor responsible for the maturation of immunological faculty. *Nature (London).* **199:** 653.

11. Law, L. W., N. Trainin, R. H. Levey, and W. F. Barth. 1964. Humoral thymic factor in mice: Further evidence. *Science (Washington)*. **143:**1049.
12. Osoba, D. 1965. The effects of thymus and other lymphoid organs enclosed in Millipore chambers on neonatally thymectomized mice. *J. Exp. Med.* **122:**633.
13. Aisenberg, A. C., and B. Wilkes. 1965. Partial immunological restoration of neonatally thymectomized rats with thymus-containing diffusion chambers. *Nature (London)*. **205:**716.
14. Sherman, J. D. 1967. Thymus humoral factor. Demonstration and influence on homograft rejection in the hamster. *Arch. Pathol.* **83:**251.
15. French, C. A. H., J. W. Watson, F. C. Walker, P. S. Gardner, and C. A. Green. 1966. Evidence for a humoral thymic factor in rabbits. *Immunology*. **10:**187.
16. Metcalf, D. 1956. The thymic origin of the plasma lymphocytosis stimulating factor. *Brit. J. Cancer*. **10:**442.
17. de Somer, P., P. Denys Jr., and R. Leyten. 1963. Activity of a non-cellular calf thymus extract in normal and thymectomized mice. *Life Sci.* **11:**810.
18. Klein, J. J., A. L. Goldstein, and A. White. 1965. Enhancement of *in vivo* incorporation of labeled precursors into DNA and total protein of mouse lymph nodes after administration of thymic extracts. *Proc. Nat. Acad. Sci. (U.S.A.)*. **53:**812.
19. Trainin, N., A. Bejerano, M. Strahilevitch, D. Goldring, and M. Small. 1966. A thymic factor preventing wasting and influencing lymphopoiesis in mice. *Israel J. Med. Sci.* **2:**549.
20. Trainin, N., and M. Linker-Israeli. 1967. Restoration of immunologic reactivity of thymectomized mice by calf thymus extracts. *Cancer Res.* **27:**309.
21. Trainin, N., M. Burger, and M. Linker-Israeli. 1967. Restoration of homograft response in neonatally thymectomized mice by a thymic humoral factor. *In* Advances in Transplantation. Proceedings of the 1st International Congress of the Transplantation Society. Munksgaard, Copenhagen. 91.
22. Small, M., and N. Trainin. 1967. Increase in antibody-forming cells of neonatally thymectomized mice receiving calf thymus extract. *Nature (London)*. **216:**377.
23. Law, L., and H. D. Agnew. 1968. Effect of thymic extracts on restoration of immunologic competence in thymectomized mice. *Proc. Soc. Exp. Biol. Med.* **127:** 953.
24. Miller, J. F. A. P. 1960. Studies on mouse leukemia. The role of the thymus in leukemogenesis by cell-free leukemic filtrates. *Brit. J. Cancer*. **14:**93.
25. Auerbach, R., and A. Globerson. 1966. *In vitro* induction of the graft-versus-host reaction. *Exp. Cell Res.* **42:** 31.
26. Simonsen, M., J. Engelbreth-Holm, E. Jensen, and H. A. Poulsen. 1958. A study of the GvH reaction in transplantation to embryos, F_1 hybrids and irradiated animals. *Ann. N.Y. Acad. Sci.* **73:**834.
27. Simonsen, M. 1962. Graft versus host reactions. Their natural history and applicability as tools of research. *Progr. Allergy*. **6:**349.
28. Dalmasso, A. P., C. Martinez, and R. A. Good. 1962. Failure of spleen cells from thymectomized mice to induce graft vs. host reactions. *Proc. Soc. Exp. Biol. Med.* **110:**205.
29. Yunis, E. J., C. Hilgard, C. Martinez, and R. A. Good. 1965. Studies on immunologic reconstitution of thymectomized mice. *J. Exp. Med.* **121:**607.

30. Rieke, W. O. 1966. Lymphocytes from thymectomized rats: Immunologic proliferative and metabolic properties. *Science (Washington)*. **152**:335.
31. Davies, A. V. S., E. Leuchars, V. Wallis, R. Marchant, and E. V. Elliott. 1966. The mitotic response of thymus-derived cells to antigenic stimulus. *Transplantation*. **4**:438.
32. Claman, H. N., E. A. Chaperon, and J. C. Selner. 1968. Thymus-marrow immunocompetence III. The requirement for living thymus cells. *Proc. Soc. Exp. Biol. Med.* **127**:462.
33. Miller, J. F. A. P., and G. F. Mitchell. 1968. Cell to cell interaction in the immune response I. Hemolysin-forming cells in neontally thymectomized mice reconstituted with thymus or thoracic duct lymphocytes. *J. Exp. Med.* **128**:801.
34. Gregoire, C. 1935. Recherches sur la symbiose lymphoepitheliale au niveau du thymus de mammifere. *Arch. Biol.* **46**:717.
35. Globerson, A., and R. Auerbach. 1967. Reactivation *in vitro* of immunocompetence in irradiated mouse spleen. *J. Exp. Med.* **126**:223.