SEROLOGICAL ANALYSIS OF THYMUS AND SPLEEN GRAFTS*

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Thymic lymphocytes are continuously replaced by stem cells entering the thymus. The thymus of animals receiving lethal total body irradiation, followed by administration of bone marrow or spleen cells, is repopulated by cells derived from the inoculum (1–6). Similarly, repopulation of the unirradiated thymus eventually occurs in animals in which only the lower third of the body was irradiated prior to bone marrow administration (7). The thymus of mice (8) or of chicken embryos (9) joined by parabiosis contains cells derived from the partner. Analysis of thymus grafts has shown that the lymphoid elements are replaced by cells derived from the host (10–12).

Thymic lymphocytes constantly display distinctive serological properties (see references 12 and 14) in spite of their replacement by the continuous entry of stem cells. Thymus cells of all strains of mice show a distinctive sensitivity to the cytotoxic effect of guinea pig serum (GPS) (15–19). The thymus cells of some strains of mice, such as the A strain, possess a thymus-specific antigen, the TL (thymus-leukemia) antigen, while other strains of mice, such as the C57BL strain, do not have this antigen on their thymus cells (20, 21).

Since the lymphoid cells of the thymus are constantly replaced by cells which lack thymus-distinctive serological properties, the thymus must be capable of endowing such properties on the entering cells. The existence of such a process was demonstrated in radiation chimeras (5). It was found that the bone marrow, spleen, and thymus of lethally irradiated C57BL mice injected with strain A bone marrow or spleen cells were repopulated by donor cells. However, thymus-distinctive serological properties appeared only in the cells lodging in the thymus, but failed to appear in cells repopulating other organs. In the present investigation, the expression of antigens was studied in cells repopulating thymus and spleen grafts.

Materials and Methods

Mice.—Donors of neonatal thymus and spleen grafts belonged to the C57BL/6 inbred strain of mice. Recipients of the grafts were male, adult (A × C57BL/6) F_1 hybrids. Some of these hybrids were thymectomized 3 wk before receiving thymus grafts. Mice belonging to the

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A, BALB/c, and C57BL/6 strains, and (BALB/c × C3H/An) F_1 hybrids were used for the preparation of isoantisera. The origin of these inbred mice was described previously.[1]

Grafts.—Thymuses and spleens were removed from neonatal donors and transferred to normal saline. A whole neonatal spleen or one thymic lobe were introduced into an 18 gauge trocar. One or both kidneys were exposed under ether anesthesia, and the content of the trocar was introduced beneath the kidney capsule. When grafts were made to both kidneys, they received either the same type of graft, or a thymus graft was implanted in one kidney and a spleen graft in the other kidney.

Serological Techniques.—

Antisera.—Isoantisera were prepared by repeated intraperitoneal administration of lymphoid cells. Mice were bled from the paraorbital venous plexus 1 wk after the last immunizing challenge. Sera were stored at −20°C. The presence of *Histocompatibility-2*^{b} (*H-2*^{b}) isoantigenicity, common to host and donor cells, was demonstrated by a serum prepared in strain A mice by immunization with C57BL spleen cells. In pilot experiments, invading host cells were detected in C57BL grafts by a serum prepared in C57BL mice by immunization with strain A spleen cells. However, this antiserum was inadequate for quantitative determinations, since it often killed only a low percentage of thymus cells of (A × C57BL) F_1 mice. A suitable reagent for detection of host cells was an isoantiserum prepared in C57BL mice by immunization with BALB/c thymus cells. This antiserum also contains, apart from antibodies against *H-2* isoantigens, antibodies against a non-*H-2* antigen having a high concentration in the thymus, spleen, and brain (22). The C57BL anti-BALB/c thymus serum is cytotoxic for thymus cells of mice of the 129 strain, which have the same *H-2* antigenicity as mice of the C57BL strain. The non-*H-2* antigen detected by this antiserum is distinct from the TL antigen, and preliminary genetic studies[2] indicate that it differs from the θ-antigens described by Reif and Allen (23).

TL antigenicity was determined by an antiserum prepared in (BALB/c × C3H) F_1 hybrid mice by immunization with thymus cells of mice of the A strain.

Cytotoxicity test.—The modification of Boyse et al. (24) of the cytotoxicity test of Gorer and O'Gorman (25) was used. Details of the technique were described previously (17, 26). The guinea pig serum used as a source of complement in the cytotoxicity tests was absorbed with normal tissues to remove its cytotoxicity for mouse thymus cells (15–19).

Thymus and spleen grafts were carefully excised from surrounding kidney tissue at various intervals after grafting. The grafts were minced with scissors and cell clumps were broken up by drawing them up and down a Pasteur pipette. Cell suspensions were prepared in normal saline at a concentration of 1×10^6 cells/ml. Each cell suspension was studied in cytotoxicity tests with the following isoantisera: (*a*) C57BL anti-BALB/c thymus serum, (*b*) A anti-C57BL spleen serum, (*c*) (BALB/c × C3H) F_1 anti-A thymus serum. The number of cells that were nonviable after exposure to each of the isoantisera, in the presence of complement, was determined. The results were expressed as cytotoxic indices according to the formula (26):

$$\text{Cytotoxic index} = \frac{\text{Per cent nonviable (experimental)} - \text{per cent nonviable (control)}}{\text{Per cent viable (control)}} \times 100$$

The control values were those of the complement control, containing cells and complement but no isoantibody.

Cytotoxicity of guinea pig serum.—The sensitivity of each cell suspension to the cytotoxic

[1] Schlesinger, M., and D. Hurvitz. Characterization of cytotoxic isoantibodies produced in RIII mice. Submitted for publication.

[2] Schlesinger, M., and D. Hurvitz. Unpublished data.

effect of GPS was determined by adding 25,000 cells in 0.025 ml saline to an equal volume of serial two-fold saline dilutions of unabsorbed GPS. After incubation at 37°C for 1 hr, the cell viability was determined after the addition of 0.04% trypan blue.

RESULTS

Serological Analysis of Thymus Grafts.—Neonatal thymuses from C57BL mice were grafted beneath the kidney capsule of adult (A × C57BL) F_1 hosts, and their serological properties were studied at various time intervals. Cells possessing isoantigens of the host could be detected in thymus grafts as early

TABLE I

Serological Analysis of Cells of C57BL Thymus Grafts to (A × C57BL) F_1 Recipients

Days after grafting	Host cells*	TL-positive cells	Sensitivity to GPS
	%	%	
10	8, 0	0, 0	+, +‡
11	46, 20	1, 0	+, +
13	89, 75, 65, 27, 24, 11	15, 5, 3, 0, 0, 0	+, +, +, +, +, +
14	85, 40	0, 0	+, +
15	89, 85, 83, 80, 69, 48, 44, 19, 8	71, 44, 44, 18, 18, −,§ 11, 0, 0	+, +, +, +, +, +, +, +, +
16	95, 79, 72, 62, 60, 45	65, 62, 44, 32, 30, 15	+, +, +, +, +, +
17	93, 53, 52, 50, 49, 48, 44, 34	76, 71, 68, 59, 57, 51, 39, 33	+, +, +, +, +, +, +, +
18	91, 89, 87, 80	82, 66, 57, 53	+, +, +, +
19	95, 90, 89, 85, 36	74, 72, 68, 58, 53	+, +, +, +, +
20	96	76	+
27	81	65	+

* Each value represents the results obtained with a single graft.

‡ +, over 70% of the cells killed by GPS diluted 1:2 to 1:4.

§ −, not done.

as 11 days after grafting (Table I). Complete repopulation by host cells could be found in some grafts analyzed 13 days after grafting. The mean percentage of host cells invading thymus grafts gradually increased between the 11th and 18th day after grafting (Fig. 1). Grafts examined 17 days after grafting showed some resistance to the cytotoxic effect of isoantibodies and complement. This was true both for the antiserum used to detect host cells (C57BL anti-BALB/c thymus) and for a control serum detecting *H-2* isoantigens common to host and donor tissues (A anti-C57BL spleen).

Apart from one exceptional graft, none of the thymus grafts examined between 10 and 14 days after grafting contained any significant TL antigenicity. TL-positive cells appeared in grafts analyzed 15 days after grafting, and their

mean percentage gradually increased within the next three days (Table I, Fig. 1).

The cells residing in thymus grafts were sensitive to the cytotoxic effects of

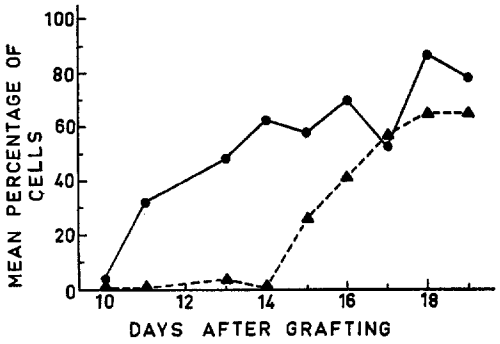

FIG. 1. Mean percentage of host cells and of TL-positive cells in C57BL thymuses at various intervals after grafting to (A × C57BL) F_1 hybrids. Circles, mean percentage of host cells; triangles, mean percentage of TL-positive cells.

TABLE II

Serological Analysis of Cells of C57BL Spleen Grafts to (A × C57BL) F_1 Recipients

Days after grafting	Host cells*	TL-positive cells	Sensitivity to GPS
	%	%	
8	70	0	−‡
10	70, 68, 63	0, 0, 0	−, −, −
11	75	0	−
13	83, 53	0, 0	−, −
15	95, 81	0, 0	−, −
16	71	0	−
17	86, 73, 73, 40	0, 0, 0, 0	−, −, −, −
18	57	0	−
19	71, 67	0, 0	−, −
21	57	0	−
23	82	0	−
26	72, 66	0, 0	−, −

* Each value represents the results obtained with a single graft.

‡ −, less than 10% of the cells killed by GPS diluted 1:2.

GPS throughout the period of observation. No significant variations in the sensitivity to GPS could be found during phases when the cells in the grafts were predominantly of donor or of host origin, or at intermediate stages.

Serological Analysis of Spleen Grafts.—Neonatal C57BL spleens were grafted beneath the kidney capsule of (A × C57BL) F_1 hosts, and analyzed at various

periods after grafting. Most of the cells residing in spleen grafts were found to be of host origin as early as 8 days after grafting, and host cells continued to constitute the majority of the cells residing in spleen grafts throughout the period of observation (Table II). At no stage could any TL-positive cells be demonstrated in splenic grafts, and at no stage did spleen grafts contain cells which were sensitive to the cytotoxic effect of GPS.

Serological Analysis of Thymus Grafts in Thymectomized Hosts.—In intact (A × C57BL) F_1 hosts bearing C57BL thymus grafts, TL-positive and GPS-sensitive cells were present in the thymus of the hosts. In order to exclude the possibility that the TL-positive, GPS-sensitive cells found in repopulated thymic grafts were derived from the thymus of the host, C57BL thymuses were

TABLE III

Serological Analysis of Cells of C57BL Thymus Grafts to Thymectomized (A × C57BL) F_1 Recipients

Days after grafting	Host cells*	TL-positive cells	Sensitivity to GPS
	%	%	
11	−, −‡	0, 0	+, +§
15	93, 89	78, 21	+, +
17	94, 93	56, 53	+, +
18	86, 69	85, 54	+, +
19	−	57	+
20	−, −	50, 44	+, +

* Each value represents the results obtained with a single graft.

‡ −, not done.

§ +, over 70% of the cells killed by GPS diluted 1:2 to 1:4.

grafted to thymectomized (A × C57BL) F_1 recipients. Cells of thymectomized hosts which repopulated thymic grafts were found to be fully capable of acquiring TL antigenicity and GPS sensitivity (Table III).

DISCUSSION

The present study confirms previous observations that thymus and spleen grafts are repopulated by host cells. Thymus-distinctive serological properties (13) were found only in the cells repopulating thymus grafts, but not in those repopulating spleen grafts.

Early attempts to demonstrate invasion of thymus grafts by host cells have utilized histological methods. The results of such studies were inconclusive due to the difficulty of distinguishing host from donor cells. Nevertheless, Grégoire, over 30 yr ago, was convinced from histological studies of thymus grafts in guinea pigs that these were invaded by host cells (27). Clear demon-

stration of the repopulation of thymus and spleen grafts by host cells has been achieved in recent years through various experimental approaches:

1. Leukemias developing in thymuses grafted to thymectomized animals were analyzed by transplantation to various secondary hosts or by cytological analysis. Some of these leukemias were found to be composed of cells derived from the primary host, rather than from the donor of the thymus graft (28).

2. Chromosome analysis of dividing cells may enable distinction between host and donor cells, when they differ by a cytological marker. This technique enabled definite demonstration of repopulation of thymus grafts in mice (11, 12, 29, 30), and chicken (9), and of murine spleen grafts (31).

3. Repopulating host cells were identified in thymus and spleen grafts by serological analysis (10).

4. Small "lymphocyte-like" cells observed in grafts of in vitro irradiated thymuses were identified as host cells (32). Such cells appeared only in irradiated thymuses grafted to untreated recipients, but failed to appear when irradiated grafts were implanted in lethally irradiated hosts.

In the present study, spleen grafts examined 8 days after grafting were already completely repopulated by host cells, while thymus grafts were partially repopulated only 11 days after grafting. Complete repopulation could be found in some of the thymus grafts examined 13 days after grafting. The earlier repopulation of spleen grafts as compared to thymus grafts may be related to a phenomenon observed by Balner and Dersjant (33). After injection of labeled bone marrow cells into irradiated isogeneic recipients, a high concentration of labeled cells was found within 2 days in the spleen of irradiated recipients, while only a small number of labeled cells could be found in the thymus even as late as 8 days after their administration. The serological methods used in the present study revealed that extensive invasion of spleen grafts by host cells occurred earlier than could be detected by the chromosome marker technique. Only small numbers of dividing host cells could be found in spleen grafts during the first 9 days after grafting, and host cells constituted only a third of the dividing cells present in spleen grafts 13 days after their implantation into sham-splenectomized recipients (31). It seems, therefore, that host cells reside in spleen grafts for several days before undergoing mitosis.

While both thymus and spleen grafts are repopulated by host cells, the invading cells differentiate in different directions in the two types of grafts. Repopulated spleen and thymus grafts display all the architectural and cytological features characteristic of the tissue grafted (cf. reference 34). In spleen grafts, repopulation is not limited to lymphoid cells, but erythroid, myeloid, and megakaryocytic cells are also replaced by host cells (31). Metcalf (35) found that thymic grafts underwent involution in pregnant or testosterone-treated hosts, although to a lesser extent than the intact thymus. Harris and Ford (36) presented evidence that descendants of the host cells entering thymic

grafts are altered in a way that confers on them the capacity to seek out and proliferate selectively in lymph nodes.

In the present study, the cells of (A × C57BL) F_1 hosts invading C57BL thymus grafts acquired the thymus-specific TL antigen (20, 21) and became sensitive to the cytotoxic effect of GPS (15–19). Host cells repopulating spleen grafts showed neither of these properties. TL-positive and GPS-sensitive cells are present only in the thymus and were never found in any other organ. The possibility that the thymus of the host may supply such cells for repopulation of thymus grafts was excluded by examining grafts in thymectomized recipients.

From the reports available in the literature and from the data in the present study, a composite picture can be constructed of the sequence of events leading to renewal of thymic structure by host cells:

Invasion of neonatal thymus grafts by host cells probably occurs early after transplantation. This can be readily demonstrated in grafts of thymuses which had been irradiated in vitro. In such grafts, small lymphocyte-like cells, probably of host origin, appear 4 days after grafting, mainly in the region of the renal graft junction (32). Within a few more days, these cells increase in number and are interspersed among epithelial cells. Well-differentiated large and medium lymphocytes, probably of host origin, appear in irradiated thymus grafts within 7 days after grafting. Moore and Owen found that chick thymus grafts from 8 day embryos transplanted to the chorioallantois were fully repopulated by host cells within 4 days after grafting (9).

The serological methods employed in the present study enabled the detection of a large population of host cells in thymus grafts within 11 days after grafting, and by 13 days some grafts were completely repopulated by host cells. From the data available in the literature it seems that a significant percentage of dividing host cells may be detected in thymic grafts only at a time when, according to the present study, considerable repopulation of the grafts by host cells has already taken place. Thus Dukor et al. (12) could not detect any dividing cells in subcapsular thymus grafts examined 12 days after grafting. 13 days after grafting, only 5–9% of the dividing cells were host cells, while in grafts examined 15 days after grafting 33–73% of the dividing cells were already of host origin. Similarly, Leuchars et al. (30), analyzing thymic grafts within an isogeneic donor-host combination, found that less than 5% of the dividing cells were of host origin in grafts examined within 16 days after grafting. It seems, therefore, that the repopulation of thymus grafts by host cells does not depend to any considerable extent on the multiplication of host cells within the graft.

TL-positive donor cells can be found in the thymus of lethally irradiated C57BL recipients after the administration of bone marrow cells from strain A mice (5). It was concluded from indirect evidence that these TL-positive cells

were derived from phenotypically TL-negative cells in the inoculum. This conclusion was based on the inability to detect TL-positive cells in the bone marrow of normal strain A mice, and on the finding that in vitro (5) or in vivo (21) exposure of such bone marrow cells to antibodies against the TL antigen failed to interfere with the expression of this antigen. In the present study it was possible to demonstrate directly that the host cells that repopulate thymus grafts initially lack TL antigenicity. Invading host cells acquire the TL antigen only after residing in the thymus graft for 3–4 days. Since the cells repopulating the spleen grafts do not acquire thymus-distinctive serological properties, it seems that the reticuloepithelial elements of the graft, which are not exchanged by host cells (11), are capable of guiding and directing the differentiation of the invading host cells. The exact nature of this process is far from clear. It has previously been demonstrated in radiation chimeras that the TL antigen appears in cells entering the thymus only if the cells are derived from donors who have the appropriate genetic information (5). The mechanism underlying the appearance of the TL antigen in cells entering the thymus could therefore be an inductive process. The thymic environment may affect the genetic regulatory mechanism of the entering cells, and lead to the expression of the TL antigen on cells which have the appropriate genetic information for the antigen, but do not express it phenotypically. Similar inductive processes would lead to the expression of other thymus-distinctive serological processes in cells repopulating thymus grafts.

SUMMARY

Thymus and spleen grafts from neonatal C57BL mice were implanted beneath the kidney capsule of (A × C57BL) F_1 hybrids. At various intervals after implantation, the grafts were analyzed serologically. Cells of each graft were tested for the presence of cells of host origin, TL (thymus-leukemia) antigenicity, and sensitivity to the cytotoxic effect of guinea pig serum (GPS).

Thymus grafts showed partial repopulation by host cells 11 days after grafting, and some grafts were completely repopulated by host cells 13 days after grafting. All thymus grafts were fully repopulated 18 days after grafting. With one exception, thymus grafts contained no significant number of TL-positive cells within 14 days after grafting. TL-positive cells appeared in thymus grafts examined 15 days after implantation, and their number increased up to the 18th day after implantation. Cells residing in thymus grafts remained sensitive to GPS throughout the period of observation. The acquisition of thymus-distinctive serological properties by host cells repopulating thymus grafts was similar in intact and in thymectomized recipients.

Spleen grafts were completely repopulated by host cells as early as 8 days after grafting. The cells residing in spleen grafts remained TL-negative through-

out the period of observation, and were refractory to the cytotoxic effect of GPS.

It is thus apparent that, while both spleen and thymus grafts are invaded by TL-negative cells, only those entering the thymus acquire the antigen. The nature of the process by which the thymus endows thymus-distinctive properties on cells entering it is discussed.

BIBLIOGRAPHY

1. Ford, C. E., J. L. Hamerton, D. W. H. Barnes, and J. F. Loutit. 1956. Cytological identification of radiation-chimeras. *Nature.* **177**:452.
2. Gengozian, N., I. S. Urso, C. C. Congdon, A. D. Conger, and T. Makinodan. 1957. Thymus specificity in lethally irradiated mice treated with rat bone marrow. *Proc. Soc. Exptl. Biol. Med.* **96**:714.
3. Popp, R. A. 1961. Repopulation of thymus by immunologically competent cells derived from donor marrow. *Proc. Soc. Exptl. Biol. Med.* **108**:561.
4. Ford, C. E., and H. S. Micklem. 1963. The thymus and lymph-nodes in radiation chimeras. *Lancet.* **1**:359.
5. Schlesinger, M., E. A. Boyse, and L. J. Old. 1965. The thymus cells of radiation-chimeras: TL phenotype, sensitivity to guinea-pig serum, and origin from donor cells. *Nature.* **206**:1119.
6. Micklem, H. S., C. E. Ford, E. P. Evans, and J. Gray. 1966. Interrelationships of myeloid and lymphoid cells: studies with chromosome-marked cells transfused into lethally irradiated mice. *Proc. Roy. Soc. London, Ser. B.* **165**:78.
7. Ford, C. E., H. S. Micklem, E. P. Evans, J. G. Gray, and D. A. Ogden. 1966. The inflow of bone marrow cells to the thymus: Studies with part-body irradiated mice injected with chromosome-marked bone marrow and subjected to antigenic stimulation. *Ann. N.Y. Acad. Sci.* **129**:283.
8. Harris, J. E., D. W. H. Barnes, C. E. Ford, and E. P. Evans. 1964. Cellular traffic of the thymus: Experiments with chromosome markers. Evidence from parabiosis for an afferent stream of cells. *Nature.* **201**:886.
9. Moore, M. A. S., and J. J. T. Owen. 1967. Experiments on the development of the thymus. *J. Exptl. Med.* **126**:715.
10. Green, I. 1964. The regeneration of F_1 host cell spleen and thymus at ectopic sites in F_1 animals induced by implantation of parental spleen and thymus. *J. Exptl. Med.* **119**:581.
11. Metcalf, D., and R. Wakonig-Vaartaja. 1964. Stem cell replacement in normal thymus grafts. *Proc. Soc. Exptl. Biol. Med.* **115**:731.
12. Dukor, P., J. F. A. P. Miller, W. House, and V. Allman, 1965. Regeneration of thymus grafts. I. Histological and cytological aspects. *Transplantation.* **3**:639.
13. Schlesinger, M. 1967. Expression of antigens in normal mammalian cells. *In* Immunity, Cancer and Chemotherapy. E. Mihich, editor. Academic Press Inc., New York. 281.
14. Schlesinger, M., and V. K. Golakai. 1967. Loss of thymus-distinctive serological characteristics in mice under certain conditions. *Science.* **155**:1114.

15. Reif, A. E. 1963. Immune cytolysis of mouse thymic lymphocytes. *J. Immunol.* **91**:557.
16. Palm, J. 1961. Immunogenetic aspects of tissue transplantation. *In* Transplantation of Tissue and Cells. R. E. Billingham and W. K. Silvers, editors. Wistar Institute Press, Philadelphia. 113.
17. Schlesinger, M. 1965. Immune lysis of thymus and spleen cells of embryonic and neonatal mice. *J. Immunol.* **94**:358.
18. Wakefield, J. D., and J. R. Batchelor. 1966. The effect of natural antibody in guinea-pig serum on mouse lymphoma cells in vitro and in vivo. *Immunology.* **11**:441.
19. Schlesinger, M., A. Cohen, and D. Hurvitz. 1966. Inhibition by carbohydrates of the cytotoxicity of heterologous sera for mouse thymus cells. *Israel J. Med. Sci.* **2**:616.
20. Old, L. J., E. A. Boyse, and E. Stockert. 1963. Antigenic properties of experimental leukemias. I. Serological studies *in vitro* with spontaneous and radiation-induced leukemias. *J. Natl. Cancer Inst.* **31**:977.
21. Boyse, E. A., L. J. Old, and E. Stockert. 1965. The TL (thymus leukemia) antigen: A review. *In* Fourth International Symposium on Immunopathology. P. Grabar and P. A. Miescher, editors. Schwabe, Basel. 23.
22. Schlesinger, M., and D. Hurvitz. 1967. Antibodies against non- H-2 isoantigens in C57BL mice immunized with allogeneic thymus cells. 1st International Congress Transplantation Society, Paris. 192. (Abstr.)
23. Reif, A. E., and J. M. V. Allen. 1964. The AKR thymic antigen and its distribution in leukemias and nervous tissues. *J. Exptl. Med.* **120**:413.
24. Boyse, E. Z., L. J. Old, and E. Stockert. 1962. Some further data on cytotoxic isoantibodies in the mouse. *Ann. N.Y. Acad. Sci.* **99**:574.
25. Gorer, P. A., and P. O'Gorman. 1956. The cytotoxic activity of isoantibodies in mice. *Transplant. Bull.* **3**:142.
26. Hattler, B. G., M. Schlesinger, and D. B. Amos. 1964. The differing survival of normal and sensitized spleen cells transferred to allogeneic hosts. *J. Exptl. Med.* **120**:783.
27. Grégoire, C. 1935. Recherches sur la symbiose lymphoepitheliale au niveau du thymus de mammifere. *Arch. Biol. Liege.* **46**:717.
28. Miller, J. F. A. P. 1962. Role of the thymus in virus-induced leukemia. *In* Ciba Foundation Symposium on Tumour Viruses of Murine Origin. G. E. W. Wolstenholme and M. O'Connor, editors. Churchill, London. 262.
29. Metcalf, D., R. Wakoning-Vaartaja, and T. R. Bradley, 1965. The growth and repopulation of thymus grafts placed under the kidney capsule. *Australian J. Exptl. Biol. Med. Sci.* **43**:17.
30. Leuchars, E., A. Morgan, A. J. S. Davies, and V. J. Wallis. 1967. Thymus grafts in thymectomized and normal mice. *Nature.* **214**:801.
31. Metcalf, D., and R. Wakonig-Vaartaja. 1964. Host cell repopulation of normal spleen grafts. *Lancet.* **1**:1012.
32. Blackburn, W. R., and J. F. A. P. Miller. 1967. Electron microscopic studies of

thymus graft regeneration and rejection. II. Syngeneic irradiated grafts. *Lab. Invest.* **16**:833.

33. Balner, H., and H. Dersjant. 1964. Early lymphatic regeneration in thymectomized radiation chimeras. *Nature.* **204**:941.
34. Metcalf, D. 1966. The Thymus. Springer Verlag, Berlin.
35. Metcalf, D. 1964. Effect of pregnancy and testosterone on thymus grafts. *Transplantation.* **2**:541.
36. Harris, J. E., and C. E. Ford. 1964. Cellular traffic of the thymus: Experiments with chromosome markers. Evidence that the thymus plays an instructional part. *Nature.* **201**:884.