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Rationale for combined use of fetal liver and thymus for immunological reconstitution in patients with variants of severe combined immunodeficiency

(lymphoid differentiation/embryology/transplantation/thymic epithelium/chimerism)

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ABSTRACT Bone marrow cells from a patient with severe combined immunodeficiency were studied *in vitro* for thymus-dependent lymphocyte (T cell) differentiation by using, at varying times, thymic epithelial monolayers and culture supernatants, thymopoietin, ubiquitin, and thymic extract as inducing agents. On initial evaluation, with thymopoietin or human thymic extract, only a partial differentiation of marrow cells was achieved into cells bearing the human T cell antigenicity without the capacity to form rosettes with sheep erythrocytes, suggesting that the stem cells were defective. Two fetal liver transplantations aimed at reconstitution were unsuccessful, despite evidence of chimerism. Induction studies at that time demonstrated rosetting capacity (with sheep erythrocytes) of the patient's bone marrow cells after coculture with thymic epithelial monolayers but not with their supernatants. An 18-week fetal thymus (irradiated) was then transplanted, but the transplantation was unsuccessful and no clear evidence of chimerism was demonstrated. Subsequently, transplantation of another fetal liver resulted in chimerism and immunologic reconstitution. Serum thymic factor activity rose from 1:2 before transplantation to 1:16 after reconstitution. The combined use of fetal thymus and liver may provide effective immunological reconstitution in some variants of severe combined immunodeficiency.

Severe combined immunodeficiency disease (SCID) is a diagnostic term for a heterogeneous group of congenital disorders usually ascribed to a defect in the development or function of lymphoid stem cells (1). Such patients usually have defective development and function of both thymus-dependent lymphocyte (T cell) and thymus-independent lymphocyte (B cell) populations (2). Immunologic reconstitution in patients with this disease has been repeatedly achieved after transplantation of marrow from related, histocompatible donors (3–8). Allogeneic fetal liver has been shown to be a source of stem cells that can be used for hematologic and immunologic reconstitution in experimental animals. Furthermore, at appropriate stages of development, fetal liver cells do not induce lethal graft-versus-host disease (9–12). Even though initial efforts to use fetal liver transplants to correct combined immunodeficiency were inconclusive, initiated graft-versus-host disease, or failed to achieve immunologic constitution (13–15), recently, transplantation of fetal liver was proved to be successful in achieving sustained immunological reconstitution in a limited number of patients with SCID for whom no histocompatible marrow donors were available (16–20). The overall experience with fetal liver transplantation, however, has included inconsistency of engraftment and highly variable results in terms of functional reconstitution. One of several hypotheses raised to explain these inconsistent results is the possibility that replacement of lymphoid stem cells alone is not sufficient for functional reconstitution in all cases of SCID. This possibility was emphasized by the recent studies of Pyke *et al.* (21) demonstrating defective functional development of the thymus *in vivo* in one patient with a variant of SCID.

In this report, we present evidence to indicate that, in certain variants of SCID, transplantation of both fetal liver and thymus may be necessary to provide both the stem cell and an inductive influence necessary for immunologic reconstitution.

MATERIALS AND METHODS

Case Report. The patient, a 20-month-old white boy, was a full-term 3.8-kg product of a normal pregnancy. Growth and development were normal until age 10 weeks, at which time a monilial rash was first noted behind his right ear; this was followed by severe oral moniliasis at age 4 months. Both conditions were refractory to antifungal therapy. At 5 months of age, poor feeding and progressive weight loss developed. On Sept., 18, 1975, at age 7 months, he was admitted to our hospital.

At the time of admission, the patient was markedly cachectic, weighed 5.5 kg, and had severe moniliasis involving the oral cavity and perineal area. Clinical and roentgenographic evidence of interstitial pneumonia, subsequently documented to be due to *Pneumocystis carinii*, was noted. No thymus shadow was demonstrated on the chest x-ray. Immunologic studies revealed a lymphocyte count of $1000/mm^3$, with 72% surface immunoglobulin bearing cells, predominantly of the IgG and IgM classes; C3 and Fc receptors were detected in 81% and 2.1% of the population, respectively, and only 2–5% T cells were demonstrated by the sheep erythrocyte-rosette (E-rosette) technique. The lymphocytes were unresponsive to induction of proliferation by mitogens, allogeneic cells, or a battery of antigens in standardized assay procedures (22). No production of the lymphokine leukoyte migration inhibitory factor (LMIF) was elicited in response to antigens or mitogens (23, 24). Delayed hypersensitivity skin tests to candida and tuberculin (purified protein derivative) were nonreactive. The immunoglobulin levels were: IgG, 105 mg/100 ml; IgM, <5 mg/100 ml; IgA and IgE, not detected. The blood type was O Rh^+ and isohemagglutinins were low (anti-A, 1:1; anti-B, 1:2). Antibody production was not elicited by challenges with tetanus toxoid. The HLA typing of the patient was A9 B12/A9 B18. He was maintained in germ-free isolation in a laminar flow hood with skin and gut decontamination.

On Dec. 12, 1975, he was given 2.69×10^7 fetal liver cells, derived from a fetus of 8 weeks' gestational age, intraperitoneally. However, because the viability of these cells was less than 10%, 3 weeks later a second fetal liver transplant of 4×10^9 cells derived from a fetus of 12.5 weeks' gestational age was

Abbreviations: SCID, severe combined immunodeficiency disease; T cell, thymus-dependent lymphocyte; B cell, thymus-independent lymphocyte; E-rosettes, rosettes with sheep erythrocytes; BSA, bovine serum albumin; HTLA, human T lymphocyte antigenicity.

administered intraperitoneally. Chimerism, detected by HLA typing consistent with parental haplotypes of the donor fetal tissues, was first noted on Dec. 1, 1975, and persisted through Jan. 16, 1976; however, there was no evidence of immunologic reconstitution. On Feb. 4, 1976, an irradiated fetal thymus derived from an 18-week-old fetus was transplanted into the right quadriceps femoris. Again, no evidence of immunologic reconstitution was noted. On Mar. 30, 1976, a third fetal liver transplant of 5.87×10^7 cells from a 9.5-week-old fetus was administered intraperitoneally. The HLA type of the parental donor of the fetus was A2, AW30/B8,BW17. Within 3 weeks, evidence of chimerism was detected with an increase in lymphocyte count. On day 40, a generalized maculopapular rash developed and persisted for 24 hr.

On May 2, 1976, the responses to phytohemagglutinin P first became positive, and IgM levels rose to 12 mg/100 ml. By June 30, 1976, the proliferative responses to other mitogens rose to within normal limits. Immunoglobulin values at that time were: IgG, 180 mg/100 ml; IgM, 10–15 mg/100 ml; IgA, not detectable; and IgE, 44 μg/ml. Recontamination was initiated on June 16, 1976, and was well tolerated. The patient was discharged from the hospital on July 9, 1976.

He has been home for 8 months and has been free of infections. Growth and development have been normal. He demonstrates persistent chimerism, evidenced by strong reactions of patient's cells with antisera to A1, A2, B8 antigens, and normal cell-mediated responses *in vitro*. Immunoglobulin levels at last evaluation were: IgG, 192 mg/100 ml; IgM, 15 mg/ml; IgA, 0 mg/100 ml; and IgE, 10 units/ml.

Cultures of Thymic Epithelium. Thymic tissues were obtained from young children undergoing cardiac surgery who routinely have part of the thymus removed to permit maximum operative exposure. Thymic tissue from a 4-month-old child was cut into small pieces, teased with forceps, and cultured in tissue culture flasks or small petri dishes in RPMI 1640 medium supplemented with 30% (vol/vol) heat-inactivated fetal calf serum, gentamicin (4 μg/ml), and amphotericin B (1 μg/ml) in an atmosphere of 5% CO_2/95% air in 100% humidity as described by Pyke *et al.* (25). The culture medium was changed every week, and the supernatants from the cultures were collected, centrifuged, Millipore-filtered, and stored at −20°. After 4 weeks in culture, the epithelial monolayers were tested for E-rosetting cells prior to their use as inducers of differentiation. Fibroblast monolayers from fetal kidney and its supernatant (also stored at weekly intervals at −20°) were used as controls.

Precursor Cell Isolation. Small volumes of bone marrow cells from a normal volunteer and from the patient with SCID were aspirated from several sites on the iliac crest and defibrinated with glass beads. Mononuclear cells were initially separated by Ficoll-Hypaque gradients ($400 \times g$ for 30 min at 23°). Cells at the interface were washed twice with RPMI 1640 containing 1% (vol/vol) solution of antibiotics, suspended in 17% (wt/vol) bovine serum albumin (BSA) layered on top of a discontinuous BSA gradient (19%, 21%, 23%, 25%, and 27%), and centrifuged at 10° for 30 min at $840 \times g$ according to the procedure described by Incefy *et al.* (26). Each of the five layers was washed twice with RPMI 1640 before culturing with the inducing agents.

Inducers of Differentiation. Monolayers of cultured human thymic epithelium and their supernatants were prepared as described above. The purified polypeptides thymopoietin and ubiquitin were isolated from bovine thymus by G. Goldstein (27, 28). Partially purified human thymic extracts were prepared according to the procedure of Hooper *et al.* (29) and the protein content of these preparations (fractions 3 and 5) was determined with BSA as a standard (30).

Table 1. Differentiation of marrow cells by thymic hormones *in vitro* in SCID patient before transplantation

Additions	Cytotoxic index, by gradient layers, % HTLA⁺ cells: I	II	III	IV	V
None	7	59	27	1	2
HT-F5*	43	44	21	19	10
Thymopoietin	13	6	28	9	1
Ubiquitin	—	17	37	—	1

Marrow cells were fractionated on a BSA discontinuous density gradient and incubated for 15 hr with inducers.
*HT-F5 = human thymic extract, fraction 5 (29).

Induction of Markers. Cells obtained from each gradient layer were resuspended at a concentration of 3×10^6/ml in RPMI 1640 medium containing penicillin (50 units/ml) and streptomycin (50 μg/ml) and cocultured with thymic epithelium, thymic culture supernatant, or thymic extracts thymopoietin, and ubiquitin for 15 hr at 37° in a humidified 5% CO_2/95% air incubator. After incubation, cells were washed twice and their viability was assessed by trypan blue exclusion test. The cells were tested for ability to form E-rosettes as described by Bentwich *et al.* (31). The human thymic lymphocyte antigenicity (HTLA) markers were determined by a microcytotoxicity test in the presence of a specific antiserum against human T cells and complement as described previously (32).

RESULTS

Incefy *et al.* (26) have previously demonstrated that fraction III and, to some extent, fraction II of normal bone marrow cells from healthy volunteers are inducible for both HTLA and E-rosette markers when cocultured with thymic extracts or thymopoietin.

As shown in Table 1, in this SCID patient, cells from layers I and IV could be induced to bear HTLA markers by human thymic extract (fraction 5) and by thymopoietin. Ubiquitin appeared to induce this marker only in cells of layer III. No receptors for E-rosette formation could be induced in these same marrow cells after 15 hr of incubation with these inducing agents.

When cocultured for 15 hr with thymic epithelium and its supernatant, normal bone marrow cells developed the capacity to form E-rosettes in layers II, III, IV, and V (Table 2). Results from coculture of cells from the SCID patient with thymic epithelium and supernatant also are shown in Table 2. These studies were done after two unsuccessful fetal liver transplantations. Marrow cells from layers IV and V developed the capacity to form E-rosettes when cocultured with thymic epithelium. By contrast, supernatant from thymic epithelium cultures did not induce this marker in the patient's marrow cells.

DISCUSSION

The patient under study represented a variant of severe combined immunodeficiency in which some differentiation of B cells was clearly evident despite profound hypogammaglobulinemia and the absence of antibody responses. This case also differed from the classical form of SCID in that T-cell precursors were demonstrable in the marrow prior to transplan-

Table 2. *In vitro* differentiation of cells by thymic epithelium and thymic supernatant

Additions	% large E-rosettes by gradient layer			
	I & II	III	IV	V
	Normal marrow cells			
Control	0	0	<1	2
Thymic epithelium cell monolayers	5	4	8	1
Supernatant of thymic epithelium	3	7	13	11
Thymic epithelium cell monolayer & supernatant of thymic epithelium	5	4	10	5
	Marrow cells from SCID patient			
Control	0.3	0	0	0.3
Thymic epithelium cell monolayer	1.3	0.7	5.3	8.0
Supernatant of thymic epithelium	1	0	0.7	0
Fibroblast cell monolayer	0	0	0	N.D
Supernatant of fibroblast	0.3	0	0.7	0

Marrow cells from each gradient layer were incubated for 15 hr with inducing agents. N.D., not done.

tation and were capable of a partial differentiation restricted to induction of the HTLA marker upon exposure to thymic extracts or thymopoietin (33). We thus postulated that, in this variant of SCID, lymphoid precursors were present but their differentiation was blocked, most likely because of defects intrinsic to the stem cell. In agreement with this were the observations that a fetal thymus transplant alone failed to produce reconstitution and that eventual reconstitution of T-cell function was mediated by fetal liver-derived rather than host-derived lymphocytes.

However, the studies presented herein also serve to highlight defects of thymic function that also existed in this patient. These deficiencies are apparently extrinsic to the lymphoid stem cell. The clinical and experimental data supporting this conclusion may be summarized as follows. (*i*) Engraftment of fetal liver cells alone failed to produce functional reconstitution *in vitro* or *in vivo*. (*ii*) Prior to thymus transplantation, at a time when the patient was chimeric with lymphoid precursors from the second liver transplant, *in vitro* differentiation of marrow lymphoid precursors to populations capable of forming spontaneous E-rosettes could be achieved by incubating these cells on a normal thymic epithelium. (*iii*) Serum samples taken prior to transplantation of the thymus revealed absence of a thymic-derived factor (34) that may contribute to T-cell differentiation (35); these levels rose to normal after the thymic transplantations that apparently led to T-cell and partial B-cell activity. (*iv*) The ultimate reconstitution of T-cell function mediated by differentiated lymphoid precursors derived from the third fetal liver graft was accomplished only after engraftment of the irradiated thymus from a fetus of 18 weeks' gestation.

We therefore find evidence in this case of defects in both stem cell and thymus functions. It would appear that the immunologic reconstitution achieved required transplantation of both a fetal liver stem cell population and the thymus.

The basis for the functional defects of the thymus demonstrable in this patient is unclear. The thymus might be intrinsically defective, because of either genetic abnormality or failure to undergo a critical differentiative event. Alternatively, functional differentiation may be blocked because of either a deficiency of extrinsic trophic factors necessary for development or the presence of inhibitory influences. Pyke *et al.* (21) recently demonstrated that the thymus of a patient with a variant of SCID similar in immunologic characteristics to those of our case was histologically embryonal and functionally defective *in vivo* but could develop more mature histological features and induce differentiation of lymphoid precursors normally after culture *in vitro*. Such data suggest that extrathymic influences may be in part responsible for the deficits in thymus function observed *in vivo*.

Our failure to achieve reconstitution despite engraftment of differentiable lymphoid precursors from a normal fetal liver also suggests the possibility that certain patients with what is called SCID may have deficiencies correctable by thymus alone. We postulate that cellular and functional correction in such cases would be achieved only by transplantation of differentiated thymic tissue, such as an irradiated fetal thymus of 16 or more weeks' gestational age, used in the present case, or thymus tissue derived from similarly aged fetuses or children that has been maintained *in vitro* for a period sufficient to ensure loss of viable lymphocytes. We would expect transplantation of a prelymphoid thymus (less than 12 weeks' gestational age) to fail, even in those variants exclusively deficient in thymus function, because of host-mediated restriction on the development of the thymic graft. Indeed, transplantation of early fetal thymus has failed to produce reconstitution in patients with SCID except in those instances in which fetal thymus-derived lymphocytes were also engrafted. Restoration of immune function by grafts of the latter type has been incomplete and graft-versus-host disease has been significant (36). Conversely, transplantations of developmentally mature thymus tissue (after prolonged culture *in vitro* to deplete viable lymphocytes) have recently been shown to induce the functional development of host-derived lymphoid populations in certain variants of SCID (37).

The defects in stem cell and thymus development illustrated in this case may represent distinct entities. Alternatively, however, a basic defect in stem cell–thymus interaction can be postulated. The essential role of the thymus in the differentiation of the lymphoid stem cell is well recognized (38–48). However, the stem cell may play an equally important role as a trophic influence for thymus development (49). Failure of the stem cell to elaborate trophic mediators or of the thymus to develop systems receptive to such influences would result in the clinical and immunologic characteristics of SCID as seen in this patient. Thymus responsiveness to such influences might also be limited by extrinsic inhibitors or restricted to certain periods of embryogenesis. Engraftment of normal lymphoid precursors from a fetal liver source would thus not necessarily provide for a normal development of the host's embryonal thymus. Similarly, marrow transplantation alone in a patient of this type might not stimulate development of the host's thymus. In such a case, full constitution of cell-mediated immunity would be based on the engraftment of donor-derived post-thymic cells. Alternatively, it is possible that mature lymphocytes may be capable of acting as a thymus substitute in stem cell differentiation. Because patients with this type of SCID are represented among those successfully treated by transplantation of histocompatible marrow, the hypotheses raised are testable and may provide important insights into the basis of the immunological reconstitutions observed.

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1. Bergsma, D. & Good, R. A., eds. (1968) *Immunologic Deficiency Diseases in Man;* Proceedings (Birth Defects: Original Article Series, Vol. IV, No. 1), (National Foundation Press, New York).
2. Fudenberg, H., Good, R. A., Goodman, H. C., Hitzig, W., Kunkel, H. G., Roitt, I. M., Rosen, F. S., Rowe, D. S., Seligmann, M. & Soothill, J. R. (1971) *Pediatrics* **47,** 927–946.
3. Good, R. A. & Bach, F. H. (1974) *Clin. Immunobiol.* **2,** 63–114.
4. Meuwissen, H. J., Rodey, G., McArthur, J., Pabst, H., Gatti, R., Chilgren, R., Hong, R., Frommel, D., Coifman, R. & Good, R. A. (1971) *Am. J. Med.* **51,** 513–532.
5. Bergsma, D., Good, R. A., Finstad, J. & Paul, N. W., eds. (1975) *Immunodeficiency in Man and Animals* (Birth Defects: Original Article Series, Vol. XI, No. 1), (Sinauer Associates, Inc., Sunderland, MA).
6. Dupont, B., O'Reilly, R. J., Jersild, C. & Good, R. A. (1974) in *Progress in Immunology II.*, eds. Brent, L. & Holborow, J. (American Elsevier, New York), Vol. 5, pp. 203–214.
7. Good, R. A. (1973) *Harvey Lect.* **67,** 1–107.
8. Buckley, R. H. (1971) in *Progress in Immunology: International Congress of Immunology, 1st,* ed. Amos, B. (Academic Press, New York), pp. 1061–1080.
9. Uphoff, D. E. (1958) *J. Natl. Cancer Inst.* **20,** 625–632.
10. Tulunay, O., Good, R. A. & Yunis, E. J. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 4100–4104.
11. Yunis, E. J., Fernandes, G., Smith, J. & Good, R. A. (1976) *Transplant. Proc.* **8,** 521–525.
12. Lowenberg, B. (1975) in *Fetal liver cell transplantation, role and nature of the fetal hematopoietic stem cell,* Doctoral thesis, Erasmus University (Rotterdam) Rijswijk (A.H.), (The Netherlands, Publication of the Radiobiological Institute), pp. 1–142.
13. Hong, R., Kay, H. E. M., Cooper, M. D., Meuwissen, H., Allan, M. J. G. & Good, R. A. (1968) *Lancet* **i,** 503–506.
14. Soothill, J. F., Kay, H. E. M. & Batchelor, J. (1971) in *Proceedings of the Third Sigrid Joselius Symposium,* eds. Mäkelä, O., Cross, A. & Kosunen, T. E. (Academic Press, New York), pp. 41–52.
15. Githens, J. H., Fulginiti, V. A., Suvatte, V., Schroter, G., Hathaway, W. E., Pearlman, D. S., Kay, H. E. M., Terasaki, P. I., Hill, G. J., Kempe, C. H. & Cox, S. T. (1973) *Transplantation* **15,** 427–434.
16. Keightley, R. G., Lawton, A. R. & Cooper, M.D. (1975) *Lancet* **ii,** 850–853.
17. Ackeret, C., Pluss, H. J. & Hitzig, W. H. (1976) *Pediatr. Res.* **10,** 67–70.
18. Buckley, R. H., Whisnant, J. K., Schiff, R. I., Gilbertsen, R. B., Huang, A. T. & Platt, M. S. (1976) *N. Engl. J. Med.* **294,** 1076–1081.
19. Lustig, J. V., Rieger, C. H. L. & Rothberg, R. (1976) *Pediatr. Res.* **10,** 389.
20. O'Reilly, R. J., Pahwa, R., Pahwa, S., Schwartz, S., Smithwick, E. M., Dupont, B. & Good, R. A. (1977) in *The Third Workshop of the International Cooperative Group for Bone Marrow Transplantation in Man, Tarrytown, NY,* 1976, in press.
21. Pyke, K. W., Dosch, H. M., Ipp, M. M. & Gelfand, E. M. (1975) *New Engl. J. Med.* **293,** 424–428.
22. Cunningham-Rundles, S., Hansen, J. A. & Dupont, B. (1976) *Clin. Immunobiol.* **3,** 151–194.
23. Gorski, A., Dupont, B., Hansen, J. A. & Good, R. A. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3197–3200.
24. Gorski, A., Dupont, B., Hansen, J. A., O'Reilly, R., Smithwick, E., Gorski, R. & Good, R. A. (1976) *Clin. Exp. Immunol.* **26,** 505–510.
25. Pyke, K. W. & Gelfand, E. M. (1974) *Nature* **251,** 421–423.
26. Incefy, G. S., L'Esperance, P. & Good, R. A. (1975) *Clin. Exp. Immunol.* **19,** 475–483.
27. Goldstein, G. (1974) *Nature* **247,** 11–14.
28. Goldstein, G., Scheid, M., Hammerling, U., Boyse, E. A., Schlesinger, D. H. & Niall, H. D. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 11–15.
29. Hooper, J. A., McDaniel, M. C., Thurman, G. B., Cohen, G. H., Schulof, R. S. & Goldstein, A. (1975) *Ann. N.Y. Acad. Sci.* **249,** 125–144.
30. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
31. Bentwich, Z., Douglas, S. D., Siegal, F. P. & Kunkel, H. G. (1973) *Clin. Immunol. Immunopathol.* **1,** 511–522.
32. Incefy, G. S., Boumsell, L., Touraine, P., L'Esperance, P., Smithwick, E., O'Reilly, R. & Good, R. A. (1975) *Clin. Immunol. Immunopathol.* **4,** 258–268.
33. Incefy, G. S., Grimes, E., Kagan, W. A., Goldstein, E., Smithwick, E., O'Reilly, R. & Good, R. A. (1976) *Clin. Exp. Immunol.* **25,** 462–471.
34. Dardenne, M. & Bach, J. F. (1975) in *Biological Activity of Thymic Hormones.* ed. Van Bekkum, D. W. (John Wiley & Sons, New York), pp. 235–243.
35. Incefy, G. S., Dardenne, M., Pahwa, S., Pahwa, R., Smithwick, E., O'Reilly, R. & Good, R. A. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 1250–1253.
36. Ammann, A. J., Wara, D. W., Salmon, S. & Perkins, H. (1973) *N. Engl. J. Med.* **289,** 5–9.
37. Hong, R., Santosham, M., Wissermann, H. S., Horowitz, S., Hsu, S. & Winkelstein, J. A. (1976) *Lancet* **ii,** 1270–1272.
38. Archer, O. K. & Pierce, J. C. (1961) *Fed. Proc.* **20,** 26.
39. Good, R. A., Dalmasso, A. P., Martinez, C., Archer, O. K., Pierce, J. C. & Papermaster, B. W. (1962) *J. Exp. Med.* **116,** 773–796.
40. Miller, J. F. A. P.(1961) *Lancet* **ii,** 748–749.
41. Jankovic, B. D., Waxman Chomai, B. H. & Arnason, B. G. (1962) *J. Exp. Med.* **119,** 159–176.
42. Parrott, D. M. V. (1962) *Transplant. Bull.* **29,** 102–104.
43. Good, R. A. & Papermaster, B. W. (1964) *Adv. Immunol.* **4,** 1–96.
44. Osoba, D. & Miller, J. F. A. P. (1964) *J. Exp. Med.* **119,** 177–194.
45. Metcalf, D. (1964) in *The Thymus in Immunobiology.,* eds. Good, R. A. & Gabrielsen, A. E. (Hoeber-Harper, New York), pp. 150–182.
46. Moore, M. A. S. & Owen, J. J. T. (1976) *J. Exp. Med.* **126,** 715–725.
47. Davies, A. J. S. (1969) *Transplant. Rev.* **1,** 43–91.
48. Stutman, O., Yunis, E. & Good, R. A. (1969) *Transplant. Proc.* **1,** 614–615.
49. Gregoire, C. (1958) *Q. J. Microscop. Sci.* **99,** 511–515.