The in vivo reconstitution of congenitally thymusless mice
by Dale Darwin Isaak

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE in Microbiology
Montana State University

Abstract:
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In contrast, the ability of nude mice to respond to sheep red blood cells or to reject homografts was not generated following the intraperitoneal implantation of millipore diffusion chambers containing thymus glands, pregnancy, or the injection of the polyanions dextran SO4 or poly acrylic acid. These experiments show that the immune impairment of nude mice can be repaired by thymus derived cells, but not by soluble factors of thymic origin or polyanionic chemicals. These results suggest that the site of action of such soluble factors and polyanionic chemicals is a thymus-derived cell rather than a cell derived directly from the bone marrow.

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Signature Dale Darwin Isaak

Date May 29th, 1973

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Approved:

William G. Walter
Head, Major Department

Norman D. Reed
Chairman, Examining Committee

Henry L. Parsons
Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

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ABSTRACT

Studies presented here confirm that congenitally athymic (nude) mice are deficient in their ability to produce plaque-forming cells, rosette-forming cells and serum antibodies specific for sheep red blood cells. They are also unable to reject skin homografts. These immune deficiencies were corrected by implanting neonatal Balb/c thymus glands into nude mice.

In contrast, the ability of nude mice to respond to sheep red blood cells or to reject homografts was not generated following the intraperitoneal implantation of millipore diffusion chambers containing thymus glands, pregnancy, or the injection of the polyanions dextran SO_4 or poly acrylic acid. These experiments show that the immune impairment of nude mice can be repaired by thymus derived cells, but not by soluble factors of thymic origin or polyanionic chemicals. These results suggest that the site of action of such soluble factors and polyanionic chemicals is a thymus-derived cell rather than a cell derived directly from the bone marrow.

INTRODUCTION

Though as early as 1900, Beard had described the involvement of the thymus in the seeding of lymphocytes into the lymphoid system of animals, the role of the thymus in the proper functioning of an animal's immune system was not recognized until much later, primarily because of the failure to detect antibody production within the thymus. Askonas and White (1) described the lack of plasma cell accumulation and antibody production within the thymus, in contrast to other lymphoid tissues such as the spleen and lymph nodes, of guinea pigs immunized with ova-albumin and then assayed in vitro. Further support for the lack of a thymic involvement in the proper functioning of the immune system was inferred from results obtained by Harris et al. (2). Using adult thymectomized rabbits immunized with sheep erythrocytes (SRBC) one day following thymectomy, Harris and coworkers found no significant differences in hemolytic antibody titers in thymectomized animals and control non-thymectomized animals. Similarly, Maclean et al. (3) could find no difference between the immune responses of control rabbits and thymectomized rabbits when assayed soon after thymectomy.

Repeated clinical observations by Good (4) and Gafni et al. (5), however, strongly suggested a possible involvement of the thymus in antibody production. The repeated association of a condition characterized by total lack of antibody production, or agammaglobulinemia, and a second condition, thymoma, gave indirect evidence that impaired thymus function could lead to impaired antibody production.

Although these observations seemed to contradict the earlier results described by Harris (2) and Maclean (3), Glick and co-workers were able to partially resolve them. These studies (6) demonstrated that following the early surgical removal of the bursa of Fabricius, a lymphoid organ associated with the dorsal part of the cloaca in chickens, decreased antibody production to S. typhimurium occurred in later life. Similarly, Mueller, Wolfe and Meyer (7) were able to show decreased antibody production in chickens antigenized with bovine serum albumin, after the administration of 19-nortestosterone, a hormone which, when injected into the egg on the fifth day of incubation, causes a reduction in the size of the spleen and the thymus and a complete absence of the bursa. These observations stimulated renewed interest in the immunological role of the thymus. Experiments involving the early surgical removal of the thymus provided striking results.

In contrast to the lack of obvious immediate effects of adult thymectomy on the immune system as reported by Harris et al. and Maclean et al., neonatal thymectomy yielded animals with severely impaired immune systems. Miller (8) reported that mice thymectomized within 16 hours of birth were unable to reject homografts normally, showed a severe depletion of circulating peripheral lymphocytes along with an acute lack of germinal centers, and showed a reduced number of plasma cells. Similarly, Martinez et al. (9) reported the prolonged survival

of homografts in mice thymectomized within 24 hours of birth but not in mice thymectomized at 30 days of age. Associated with these immunological impairments in the absence of the thymus, Martinez and coworkers also reported a drop in the number of peripheral lymphocytes.

Although Miller's initial observations concerning the impaired immune response following neonatal thymectomy gave strong evidence for the importance of the thymus to the immune system, his observations concerning the severe depletion of circulating lymphocytes gave more direct evidence as to the role of the thymus in the immune system. Histological examinations of thymic tissue (10) revealed lymphocytic mitotic indexes to be 10 times those of subcutaneous lymph nodes and 5 times those of Peyer's patches and mesenteric lymph nodes. Similarly, using DNA turnover as an index of cellular proliferation, Andreasen and Ottesen (11) concluded that the greatest degree of lymphopoiesis takes place in the thymus, except in old age when the organ atrophies. Though many of these newly formed small lymphocytes are destined to die locally, others emigrate from the thymus. Murray and Woods (12), after injecting the thymus glands of guinea pigs with tritiated thymidine, looked for labeled lymphocytes in peripheral lymphoid tissue. Heavy labeling was associated with the mesenteric lymph nodes and the spleen, implying that a normal thymic function may be to generate lymphocytes which migrate to the peripheral lymphoid tissues.

A number of procedures are available for depleting animals of lymphocytes. When assayed these animals show varying degrees of immunological impairment. McGregor and Gowans (13) have shown that the removal of small lymphocytes via chronic drainage from a thoracic duct fistula severely impaired the ability of rats to respond to SRBC and tetanus toxoid. Similarly, Dougherty (14) has described the action of numerous chemicals and their role in causing the involution of lymphoid tissue and the disappearance of peripheral circulating lymphocytes. Jutila (15) reported that mice, given .2 mg. of cortisol acetate as neonates suffered a severe involution of lymphoid tissues, including the thymus and the spleen, and a decreased ability to respond to SRBC. A third method of depleting animals of their lymphoid cell populations has already been alluded to in the neonatal thymectomy work of Miller (8) which showed that in sham thymectomized mice the lymphocyte to polymorphonuclear leukocyte ratio increased progressively during the first 8 days after birth when near adult ratios were obtained. In mice thymectomized at birth, however, this ratio did not increase due primarily to an acute lymphopenia. Similarly, Mitchell and Miller (16) reported that thoracic duct cannulation of neonatally thymectomized mice yielded only 3-4% of the lymphocytes drained after 48 hours from sham operated mice, implying a severe reduction in the population of circulating small lymphocytes.

In addition to a severe reduction in the number of circulating small lymphocytes, Parrot et al. (17) have also given evidence for cellular depletion of peripheral lymphoid tissues in neonatally thymectomized mice. Histological examination of peripheral lymphoid tissues from these animals revealed a marked lymphocyte deficiency in the lymphocytic fields of the lymph nodes and the periarteriolar lymphocyte sheath of the spleen, the so called thymus-dependent areas.

Associated with the loss of the circulating pool of small lymphocytes in thymectomized animals are a number of immunological defects. Antibody production following antigenization of neonatally thymectomized animals has led to conflicting results, depending on the antigens used. Osoba and Miller (18) summarize that lower than normal levels of antibody are produced in neonatally thymectomized animals when immunized with SRBC, T_2 coliphage, diphtheria toxoid, ovalbunim, and certain serum proteins such as human gamma globulin and bovine serum albumin. On the other hand, neonatally thymectomized animals immunized with such antigens as MS-2 bacteriophage, ferritin and pneumococcus III capsular polysacharide yielded responses equal to their nonthymectomized counterparts. These conflicting results have led to classification of antigen into two groups, the thymus-dependent antigens and the thymus-independent antigens.

In addition to the immunological impairment associated with their responses to thymus-dependent antigens, neonatally thymectomized

animals have also been shown to be severely impaired with regard to cell-mediated immune responses such as the ability to induce graft-versus host reactions and the ability to reject homografts of both normal and malignant tissue. Good et al. (19) and Dalmasso et. al.(20) have shown that lymphoid cells from neonatally thymectomized mice are less able to induce graft-versus-host reactions than are similar cells from nonthymenctomized animals. In rats, Rieke (21) has observed that whereas two million thoracic duct lymphocytes from normal rats will induce a graft-versus-host reaction in an appropriate newborn host, as many as twenty million thoracic duct lymphocytes from neonatally thymectomized animals will not.

Mice thymectomized at birth, when grafted with either closely related skin (19) or with skin with major histocompatability differences, have been shown to be unable to reject the skin normally. Grafts either remained intact until the death of the animal or were rejected after prolonged survival periods. Fisher and Fisher (22) have shown that rats thymectomized at birth also have an impairment of their homograft rejection mechanisms.

Neonatally thymectomized mice have also been shown to be unable to reject tumor homografts. Martinez et al. (23) have described the inability of neonatally thymectomized mice to reject an allogeneic mammary adenocarcinoma of mouse origin, as opposed to their sham

thymectomized counterparts which rejected the tumor. Also, Osoba and Auersperg (24) have reported the establishment of a human cervical carcinoma in mice thymectomized at birth but were unable to establish the tumor in nonthymectomized controls.

Animals thymectomized as adults also show varying degrees of immunological impairment analogous to neonatally thymectomized animals but only under specific conditions. These animals, when challenged soon after surgery, were found to be immunologically capable of producing normal amounts of specific antibody when challenged with SRBC (25). That the thymus is still effective in lymphopoiesis in adult life, however, is strongly suggested by the studies of Cross et al. (26). Cross and co-workers reported that adult thymectomized mice exposed to a sublethal dose of irradiation exhibited a much prolonged recovery time as compared to non-thymectomized control animals. Similarly, adult thymectomized animals exposed to a potentially lethal dose of irradiation and subsequently protected with bone marrow cell inocula were rendered permanent immunological cripples. Miller and Osoba (18) summarized that the thymus is initially responsible during fetal life for the establishment of the long-lived recirculating population of immunologically competent small lymphocytes, and that even in adult life the thymus is necessary to maintain this population of cells.

A number of techniques, including the transplantation of thymic tissue, the inoculation of cell suspensions from various lymphoid sources, the administration of soluble thymic extracts, and the use of various chemicals have been employed to correct the immunological defects associated with neonatally thymectomized animals and adult thymectomized irradiated animals. Miller (27) has shown that the immunological defects associated with neonatally thymectomized animals can be corrected by thymus organ grafts. Thymus grafted animals recover their ability to reject homografts in both first and second set fashion and also their lymphoid tissues appear normal with the spleen, Peyer's patches and lymph nodes being well developed. Ducor et al. (28) have reported that in histological preparations thymic implants appeared to have regained normal thymic architecture within one week. The implant consisted largely of donor type cells until about two weeks when a replacement of donor cells by recipient lymphoid cells was observed. That these host type cells found in the implants are really lymphoid precursoral cells sequestered from the circulation by the thymus is suggested by the use of the T6T6 chromosomal marker in thymus grafting work (29), the use of radiation chimeras (30), and in parabiont studies (31). Dalmasso et al. (32), using spleen assays allowing them to distinguish host cells from donor cells in neonatally thymectomized mice grafted with allogeneic thymus tissue, showed that

host cells primarily were responsible for immunological activity in these mice, suggesting that the grafted thymus acts to mature the host lymphoid precursoral cells which passed through it and then out into the peripheral lymphoid system.

Dalmasso et al. (32) also give evidence that lymph node cell inocula and spleen cell inocula were effective in preventing wasting syndromes associated with neonatal thymectomy and in restoring immunological competence as measured by rejection of skin homografts and the ability to induce graft-versus-host disease, presumably because these cell inocula contained immunologically mature lymphocytes of donor thymus origin. In contrast to the effectiveness of lymph node and spleen cell preparations in establishing immune competence, thymus cell preparations were much less effective, suggesting that full immune competence of thymus derrived lymphocytes is not reached until they leave the thymus.

In addition to the cellular contribution of the thymus, a number of investigators have looked at the possibility of a thymus-derived soluble substance being involved in the immunological reconstitution of neonatally thymectomized mice. Levey, Trainin and Law (33) reported that the implantation of cell-impermeable millipore diffusion chambers (MDC) containing neonatal thymus glands into the peritoneal cavity of neonatally thymectomized mice would prevent wasting

syndromes and would establish near normal levels of peripheral blood lymphocytes and normal numbers of lymphocytes in lymphoid tissues, including the spleen, Peyer's patches and lymph nodes. Osoba and Miller (34) reported that neonatally thymectomized mice bearing MDC containing newborn thymus tissue were capable of rejecting skin homografts, whereas their non-implanted counterparts were not. Further work by these investigators (34) confirmed that neonatally thymectomized mice bearing MDC containing thymic tissue did not loose weight, were able to produce normal levels of 7S agglutinins to SRBC, rejected skin homografts within 25 days and showed varying degrees of lymphoid cellular reconstitution in lymph nodes, spleen and Peyer's patches. Their neonatally thymectomized non-implanted counterparts showed none of these characteristics. Histological examination of the thymic tissue in the MDC at the termination of the experiment revealed only fibroblasts, fibrous tissue and epithelial cells. These epithelial cells were postulated to have produced some hormonal substance that acted on lymphoid precursoral cells present in the tissues of neonatally thymectomized mice to trigger their differentiation into immunologically competent cells.

Osoba (35) has also described the reconstituting effect of this thymic hormone in a second system. Neonatally thymectomized female mice were raised to adulthood and bred. After delivering, these mice were

assayed and found capable of rejecting skin homografts and of producing anti-SRBC responses. Neonatally thymectomized females which had never given birth were found to be immunologically incompetent in each of these assays. Presumably the transplacental passage of some thymic humoral factor from the developing fetuses to the mother was responsible for her newly acquired immunological competence.

Attempts to reconstitute neonatally thymectomized animals with thymus extracts stem from an early report by Metcalf (36) in which he described the action of a lymphocytosis stimulating factor obtained from a thymus extract. De Somer et al. (37) have reported that in neonatally thymectomized mice, a single intraperitoneal (I.P.) injection of calf thymus extract (CTE) restored a normal white blood cell count within three days and prevented wasting syndromes. In addition to the establishment of normal peripheral blood pictures, CTE has also been found to restore skin and tumor homograft immunity in neonatally thymectomized mice and in adult thymectomized irradiated mice (38). Small and Trainin (39) also reported that CTE partially restored the ability of neonatally thymectomized mice to form antibody in a primary response against SRBC.

Chemical reconstitution attempts involving polynucleotides such as polyadenylic-polyuridylic acid (poly A:U) have stemmed from early work implicating the importance of an RNA and or RNA-antigen

complex involved in the activation of lymphocytes. Fishman and Adler (40, 41) isolated an RNA-rich fraction from immune macrophages which could, in vitro, cause nonimmunized lymphocytes to undergo blast transformation and to begin to synthesize antibody specific for the bacteriophages used to sensitized the macrophages. Braun and Cohen (42) postulate that the uptake of this activating RNA by lymphocytes may be controlled by specific antibody like receptor sites on lymphocytes. The antigen antibody-like reaction between lymphocyte-bound antibody and the antigen fragments detected in the RNA fraction (43) may serve to facilitate the entry of the RNA into the lymphocytes. Cone and Johnson (44) reported that the administration of poly A:U to neonatally thymectomzied mice restored their ability to produce rosette forming cells (RFC) specific for SRBC and to reject homografts, presumably because of latent thymus cell (T cell) activation by the poly A:U. Diamanstein et al. have also demonstrated an adjuvant effect of polyanionic chemicals such as polyacrylic acid (PAA) (45) and dextran SO_4 (46), which they found to increase the plaque-forming cell (PFC) response and the hemolytic antibody titers of mice challenged with SRBC. Also, they report that PAA and dextran SO_4, when administered to adult thymectomized, lethally irradiated mice, would reconstitute their response to SRBC (47).

Although studies involving characterization of T-cell depleted animals and attempts to reconstitute them have yielded much information

about the normal functioning of the thymus, there are a number of drawbacks involved in the use of T-cell depleted animals. Humphrey et al. (48) have suggested the possibility that early in the ontogeny of the animal, various cell clones are seeded from the thymus so that prior to the time of parturation the animal may have present in its peripheral lymphoid system small numbers of thymus-derived cells. Similarly, the use of adult thymectomized, lethally irradiated, bone marrow reconstituted animals has the dual disadvantage of T-cell survival following irradiation and the unavoidable introduction of low numbers of T-cells present in bone marrow preparations used in reconstitution attempts (49). Because of the presence of these T-cells, results obtained from neonatally thymectomized animals or adult thymectomized, irradiated, bone marrow reconstituted animals may not be completely valid when interpreted as having happened in an environment free of T-cells.

With these drawbacks in mind, the most useful animal for studying thymus participation in the immune response may not be the neonatally thymectomized or adult thymectomized animal, but rather the nude mouse described initially by Flanagan (50). Pantelouris (5) has characterized this mouse as being congenitally athymic in that the development of the thymus is arrested genetically at 14-15 days of in utero development, with the organs appearing as narrow strips of

tissue devoid of the lymphoid cells commonly associated with the development of normal thymus glands.

Though Raff and Wortis (52) have indicated the presence of very low numbers of thymus-derived, θpositive cells in nude mice, these mice are completely defficient in responses requiring thymus-derived lymphocytes. Responses to thymus-dependent antigens such as sheep red blood cells are very low (53, 54, 55, and 56) but responses to thymus independent antigens such as E. coli lipopolysaccharide and type III pneumococcal polysaccharide are normal (57). Also, cell-mediated immune responses are severely impaired in nude mice. Rygaard (58) has described the inability of nudes to reject either primary or secondary rat heterografts and Pantelouris (59) and Kindred (53) have given evidence that nudes fail to reject homografts. Povlsen and Rygaard (60 and 61) have reported the successful transplantation of human adenocarcinoma to nude mice and Giovanella et al. (62) have reported that human melanoma cells injected into nude mice develope into invasive tumors which are not rejected.

In addition to this lack of cell-mediated immunity, de Sousa et al. (63) have reported that nude mice have a lack of lymphocytes in the thymus-dependent areas of the peripheral lymphoid organs and a decreased number of circulating lymphocytes.

Attempts to correct these immunological deficiencies in nude mice have met with varying degrees of success. Wortis et al. (64) and

Pantelouris (65) have reported that the grafting of thymus organs will promote the rejection of homografts. Also, Kindred has reported that the injection of related thymus cells (66) or spleen, lymph node and educated thymus cells (67) will restore their ability to produce hemagglutinins and PFC specific for sheep red blood cells.

Because of the incompleteness of the data on reconstitution studies in nude mice, the following experiments were done in order to gain more information on the normal functioning of the thymus in the immune system as well as the nature of the defect in nude mice.

MATERIALS AND METHODS

Animals

Nude mice (nu/nu) and their phenotypically normal litter mates (+/nu or +/+) were the offspring of heterozygous animals obtained by crossing Re+/+nu males (supplied by R. C. Roberts and D. S. Falconer at the University of Edinburgh, Scotland) with females from our specific pathogen free (SPF) Baylor Balb/c colony and were used as the principle experimental animals throughout the study. Inbred Baylor Balb/c mice, obtained initially in 1968 from Baylor Medical School and subsequently maintained by brother-sister matings under SPF conditions were used as donors of thymus glands and lymphoid cell inocula. CBA/J mice, obtained initially in 1971 from Bar Harbor Laboratories and subsequently maintained by brother-sister matings under conventional rearing conditions, were used as donors of thymus glands and also as donors of skin in homograft immunity studies. These animals differ from the Balb/c and nude lines at the H-2 histocompatability locus.

All animals were given sterilized Purina 5010C and acidified chlorinated water (68).

Millipore Diffusion Chambers

Millipore Diffusion chambers (MDC) constructed from Scotch plastic film (number 471) and millipore membrane filters (cat.

GSWP-013-00 with 0.22 μ pore size and # VCWP-013-00 with 0.1 μ pore size) according to the method described by Bartlett and Prehn (69) were used throughout the study. Immediately preceeding implantation, the chambers were exposed to ultraviolet light at a distance of 3 inches for 7-8 minutes to reduce the microgial flora. After sealing the tape edges, the chambers were surgically implanted in the peritoneal cavity of anesthetized nude mice. A series of interrupted silk sutures were used to sew up the inner fascia and then the outer integument layers.

Chemicals

Chemicals used in attempts to stimulate antibody production included polyadenylic-polyuridylic acid (poly A:U), poly acrylic acid (PAA), and dextran SO_4. Poly A:U was formed by mixing equal volumes of polyadenylic and polyuridylic acid (lot numbers 71 and 78 respectively) obtained from Miles Laboratories Inc. and was administered intraperitoneally (I.P.) in 0.08 mg amounts immediately preceeding antigen administration. Polyacrylic acid (K and K Laboratories of Hollywood Calif. Inc.) was administer I.P. in 1.3 mg/.25ml of phosphate buffered saline amounts 2 hours preceeding the antigen. Dextran SO_4 (General Biochemicals lot number 51593, M. W. 500,000) was given I.P. in 0.5mg/.25ml phosphate buffered saline amounts ½ hour prior to antigen administration. No attempt was made to assure sterility of the solutions used.

Neonatal thymectomy

Newborn littermate animals were anesthetized by gentle cooling at -10^{o} C for 7-8 minutes in the freezing compartment of a common household refrigerator or until the animals had lost their pink color. Neonatal thymectomy was performed within the next 5 minutes by the method described by Hunter (70). Briefly, the newborns were taped to a chilled petri dish lid in crushed ice such that the head was bent sharply downward and toward the operator. A single incision through both the skin and the sternum was made to expose both glands which were then removed by gentle suction through a pasteur pipette attached to a sink aspirator. One or two interrupted silk sutures through the skin were used to close the wound and to draw the sternum together. The animals were placed under a warming light and returned to the mother following recovery from surgery. Completeness of thymectomy was determined by visual observation of the mediastinal cavity following experimental procedures.

Assays for Immunological Competence

Throughout this study, assays for the response of mice to a single dose of 1×10^{8} SRBC or 0.25 ml of a 10% suspension of SRBC consisted of the detection of specific plaque-forming cells (PFC), rosett-forming cells (RFC) and humoral antibody titers, including

hemagglutinating (HA) titers and hemolytic (HL) titers 5 days after the I.P. administration of the SRBC.

Specific PFC were detected by the slide modification of the Jerne plaque assay (71). Briefly, single celled suspensions from the spleens of immunized mice were prepared in Dutton's balanced salts. After suitable washing, varying numbers of cells were added to 0.5% agarose containing a 1:15 dilution of SRBC. Specific PFC were counted as definite zones of lyses.

Rosette-forming cells (RFC) specific for SRBC were detected by a slight modification of the technique described by Biozzi (72). Briefly, 0.1 ml of a 5% suspension of SRBC plus 0.1 ml of a mouse spleen cell preparation containing approximately 3-6 x 10^6 cells were incubated in 0.8 ml of phosphate buffered saline, at 4^o C overnight. RFC were counted the following day in a hemocytometer. Cells were considered to be positive rosette-formers when a minimum of 8 SRBC were found adhering to their surfaces. The actual number of spleen cells in the reaction mixture was then detected by lysing the red cells in 2% acetic acid. RFC were expressed as the number contained in 10^6 spleen cells.

Serum humoral hemagglutinating antibody levels were determined by the method described by Adler (73). After bleeding from the retro-orbital sinus, twofold serial dilutions of serum samples were prepared in modified barbitol buffer (74). Agglutinin (HA) titers were read

after centrifugation, following which the SRBC were resuspended and 0.1 ml of a 1:10 dilution of guinea pig complement was added to each tube. Hemolysin (HL) titers were read after 1½ hours incubation at 37° C.

In addition to the response to SRBC, immune competence was also measured as the ability to reject skin homografts. All haired recipients and donor mice were clipped and Nair was applied to remove residual hair at least 24 hours prior to grafting. Grafting procedures were adapted from Billingham and Silver (75). Briefly, full thickness grafts were prepared from previously shaved and "naired" 6 week old male CBA/J mice by skinning off the back of the donor, pinning the skin down to a disecting board and then scraping off the underlying membraneous layers. The resulting layers were then cut into circular pieces 10 mm in diameter which were then rinsed twice in phosphate buffered saline (PBS) and suspended in a final bath of PBS. Recipient mice were anesthetized with appropriate amounts of nembutal (76) and circular graft beds were cut on the dorsal area of the thorax above the rib cage. Open fit beds cut with a circular scissors were used and donor skin was placed directly on the open bed. Following the application of appropriate dressings (75), mice were allowed to recover and were returned to their cages. Casts were removed on day 8 following primary grafting procedures and on day 6 following secondary grafting procedures. Graft rejection was monitored by the development of inflamed

centers of necrosis. When the bed was 100% involved in necrosis, rejection was considered to be complete.

Thymus grafting

Twenty-four hour old Balb/c or CBA/J mice were used as thymus gland donors. Immediately preceding transplantation, donors were killed and their thymus glands were harvested into Hanks balanced salts solution. Recipients were anesthetized with nembutal and a small incision through both the dermal and the inner fascia layers was made in each axillary region. One gland was placed in each incision and 2-3 interrupted silk sutures were used to close up the incision. Animals were assayed for immune competence 2-4 weeks after thymus transplanting.

Thymus cell suspensions

Thymus cell suspensions were made by gentle screening of 24 hour old Balb/c thymus glands through stainless steel mesh until single cell suspensions were obtained in Hanks balanced salts solution. Cells were quantitated and given I.V. by tail vein injection after trypan blue exclusion viability studies were done.

RESULTS

Homograft immunity and anti-SRBC response of nudes grafted with thymus glands and sham-operated nudes.

The grafting of (CBAxAKR) F1 thymus glands to nude mice (65) has given evidence for a reconstitution effect of the glands on nude mice, but the numbers of animals involved and the results obtained did not lead to complete and satisfying conclusions. The first experiments dealing with the grafting of Balb/c thymus glands to the axillary regions of nude mice were done in an attempt to firmly establish the effect of normal thymus glands on nude mice. Experimental nude animals were given one neonatal Balb/c thymus gland in each axillary area (i.e., one donor mouse was used for each recipient) and control animals were sham operated. After a period of 2 to 4 weeks, both groups were given CBA skin grafts which differ from Balb/c at the H-2 histocompatability locus. Table I shows the results of these experiments. Animals grafted with thymus glands were able to reject the histoincompatable CBA skin in 18.7 days after primary challenge and in 10.9 days after secondary challenge, implying that an active functioning cellular immune system was operating in nudes grafted with thymus glands. As early as two weeks post operative, animals were fully capable of rejecting skin homografts as rapidly as those animals which were assayed one month following thymus gland grafting. In contrast to this, sham-operated nudes never rejected their skin homografts and all grafts remained healthy until the death of the animals or the termination of the experiment. Hair growth from the graft was taken as absolute evidence of graft acceptance. In addition to skin homograft immunity,

both experimental and control groups were assayed for the ability to produce specific PFC, RFC, and HA and HL antibodies when immunized with SRBC. When all animals had clearly rejected their grafts or had grown large patches of hair from the graft sites, 1×10^8 SRBC were administered I.P. Five days later, the animals were bled for serum, plaqued for specific PFC and assayed for specific RFC. Table I shows the results of these studies. An increase in the number of PFC is evident in the experimental group as compared to the sham-operated group. Similarly, grafted animals showed a sharp increase in the number of RFC when challenged with SRBC, as opposed to the sham-operated animals which remained at a low background level similar to that described by Reed and Jutila (57). Table I also shows that following immunization with SRBC, the experimental thymus-grafted group was able to form much higher levels of HA and HL antibodies, as compared to similarly treated sham animals.

When nude animals were grafted similarly with CBA/J thymus glands and then challenged three weeks later with CBA skin grafts, the animals were not able to reject the skin grafts, as shown in Table II. Three weeks after skin grafting, both the experimental thymus-grafted nudes and the control sham-operated nudes were immunized with 1×10^8 SRBC. Five days later, serum was harvested and titrated for both HA and HL antibody levels. Table II shows that the thymus-grafted animals

TABLE I

HOMOGRAFT RESPONSE AND ANTI-SRBC RESPONSE OF NUDES GRAFTED WITH BALB/C THYMUS GLANDS OR SHAM-OPERATED NUDES

GROUP	HOMOGRAFT SURVIVAL TIME OF DAYS		PFC/10^6		PFC/SPLEEN		SERUM Ab		RFC/10^6
	1^o	2^o	D[d]	I[e]	D	I	HA	HL	SPLEEN CELLS
THYMUS-GRAFTED GROUP[a]	(14)[b]	(10)	(9)	(9)	(9)	(9)	(9)	(9)	(6)
	18.7	10.9	260	312	18,689	21,350	812	1498	1510
SHAM OPERATED NUDES	(12)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(4)
	37.1[c]	21.6[c]	37	27	2463	1913	36	71	41

a. Animals were grafted with one neonatal Balb/c thymus gland in each axillary region. Two to four weeks later they were grafted with CBA/J skin. Following complete rejection or acceptance of the 1^o and 2^o homografts, 1×10^8 SRBC were given I.P. Five days later, specific PFC, RFC and humoral antibody levels were determined.
b. The number of animals in the group.
c. All primary and secondary skin grafts on the sham-operated nudes were healthy and had large areas of hair growth at the termination of the experiment.
d. Direct plaque forming cells.
e. Indirect plaque forming cells.

TABLE II

HOMOGRAFT RESPONSE AND ANTI-SRBC RESPONSE
OF NUDES GRAFTED WITH CBA/J THYMUS GLANDS

GROUP	No. OF ANIMALS	MEAN GRAFT SURVIVAL TIME	SERUM ANTIBODY HA	SERUM ANTIBODY HL
thymus grafted nudes[a]	5	indefinite[b]	(3)[c] 67	(3)[c] 373
sham operated nudes	3	indefinite[b]	(2) 30	(2) 120

a. Animals with either sham-operated or grafted with 24 hour old CBA/J thymus glands. Three weeks later both groups were given CBA/J skin homografts and inspected daily for signs of graft rejection.

b. None of the animals in either group showed signs of graft rejection so all surviving animals were given 1 x 10^8 SRBC. Five days later, serum was harvested and levels of specific antibodies were determined.

c. Number of animals in group.

produced higher levels of both the HA and the HL antibody types, but this increase is small when compared to the increases observed in Table I when nudes were grafted with Balb/c thymus glands.

Homograft immunity and anti-SRBC response of nude females which had given birth to thymus bearing young.

The results of attempts to reconstitute nude animals using Osoba's pregnancy system (37) involving the transplacental effect of a fetal thymic hormone on the mother are summarized in Tables III and IV. Nude females bred to either Balb/c males or littermates were immunized with 0.25 ml of a 10% suspension of SRBC within one month after delivering their wild type young. Table III shows that these animals failed to produce higher numbers of PFC/10^6 spleen cells or PFC/spleen than did similar nude mice which had never delivered thymus bearing young. Likewise, these animals did not produce higher humoral antibody titers of either the hemagglutinating or the hemolytic type than did the virgin nudes. Neither group of nudes produced PFC or humoral antibody responses comparable to those produced by littermate females immunized with similar numbers of SRBC. Nude mothers which had delivered wild type young were also incapable of rejecting CBA skin homografts when challenged within one month of delivery, as shown in Table IV. Similarly, Table IV shows that virgin nudes were unable to reject CBA skin grafts. Normal littermates of nudes, however, were able to reject

TABLE III

ANTI-SRBC RESPONSE OF VIRGIN NUDES, VIRGIN LITTER MATES AND NUDE FEMALES WHICH HAD DELIVERED WILD TYPE YOUNG[a]

GROUP	No. OF ANIMALS	DIRECT PFC/10^5	DIRECT PFC/SPLEEN	SERUM ANTIBODY HA	SERUM ANTIBODY HL
NUDES (preg.)[a]	11	18	2187	176	146
NUDES (virgin)[a]	9	16	1218	149	142
LMC (virgin)	8	170	22,323	1280	4000

a. Anti-SRBC responses of nude females delivering wild type young compared to similar responses of virgin nudes and virgin LMC. Within three weeks of delivery, experimental and control animals were immunized with .25 ml of a 10% suspension of SRBC and were assayed five days later.

TABLE IV

HOMOGRAFT RESPONSE OF VIRGIN NUDES, VIRGIN LITTERMATES AND NUDE FEMALES WHICH HAD DELIVERED WILD TYPE YOUNG[a]

GROUP	No. OF ANIMALS	MEAN GRAFT SURVIVAL TIME
NUDES (preg.)	4	Indefinite
NUDES (virgin)	5	Indefinite
LMC (normal)	6	11.0 days

a. Allograft immunity of nude females delivering wild type young compared to allograft immunity of virgin nudes and virgin LM. Experimental and control animals were grafted with six week old CBA skin within one week following delivery of thymus bearing young.

similar CBA skin grafts within 11 days after primary challenge (data from Table X).

Homograft immunity and anti-SRBC response of nude mice bearing either MDC containing neonatal Balb/c thymus glands or empty MDC

Because of the reconstitutive effects of thymic glands within cell tight millipore diffusion chambers in neonatally thymectomized animals (35, 36), it was of interest to check the effect of this diffusible thymic hormone on nude mice. Results shown in Table V were obtained from nude animals grafted I.P. with either MDC containing two neonatal Balb/c thymus glands or empty MDC. One month following the implantation of the MDC, each surviving animal was grafted with a CBA skin graft. As shown, none of the animals in either group rejected its graft and luxurious growths of hair were seen in each graft site. One month following skin grafting, the animals were sacrificed and assayed for their responses to the 1×10^8 SRBC administered 5 days earlier. As shown, the experimental group grafted with MDC containing thymic tissue was not able to produce higher numbers of specific PFC nor higher levels of HA and HL antibodies.

Neonatal thymectomy of littermates to nudes revealed that complete thymectomy within 24 hours of birth would render the animals unable to reject homografts, respond to SRBC or to maintain good health in later life. Because of difficulties with the technique, however,

TABLE V

HOMOGRAFT RESPONSE AND ANTI-SRBC RESPONSE OF NUDES GRAFTED WITH EITHER MDC CONTAINING NEONATAL THYMUS GLANDS OR EMPTY MDC[a]

GROUP	PFC/10^6 D	PFC/10^6 I	PFC/SPLEEN D	PFC/SPLEEN I	SERUM ANTIBODY HA	SERUM ANTIBODY HL	1^o HOMOGRAFT SURVIVAL
NUDES+MDC (glands)[a]	(0)[b]	(0)	(3) 2487	(3) 2560	(6) 70	(6) 70	(3) indefinite
NUDES+MDC (empty)[a]	(2) 19	(2) 22	(5) 2340	(5) 1650	(7) 17	(7) 27	(8) indefinite

a. Animals were grafted I.P. with MDC containing 2 neonatal Balb/c thymus glands or with empty MDC. Four weeks later CBA skin homografts were given and eight weeks later, 1 x 10^8 SRBC were given I.P. The animals were assayed for a five day response.

b. Number of animals in the group.

control experiments involving neonatally thymectomized littermates given MDC containing thymus tissue did not prove successful.

Because of the disparity between the effects of thymus glands within cell-impermeable MDC in the peritoneal cavity of neonatally thymectomized mice and their effects in the peritoneal cavity of nude mice, it is possible that the site of action of this thymic hormone is really immature thymus-derived cells which have been seeded from the thymus prior to the surgical removal of the thymus. Table VI shows the results of an experiment designed to test this hypothesis. Nude animals in group one were grafted I.P. with cell impermeable MDC containing two-three day old Balb/c thymus glands. These animals, when challenged two weeks later with 1×10^8 SRBC and plaqued five days later were found to be deficient in the number of specific PFC produced and also in their HA and HL antibody titers. Similarly, nudes in group three, which were given 5×10^7 Balb/c thymus cells I.V. and immunized two weeks later with 1×10^8 SRBC, did not produce high numbers of specific PFC and HA and HL antibody levels. Animals in group two were given 5×10^7 cells 24 hours following the grafting of MDC containing two thymus glands. When these animals were immunized two weeks later and assayed five days later, the results revealed that although the animals did not respond as well as litter mate

TABLE VI

THE EFFECT OF THYMIC HUMORAL FACTOR AND/OR BALB/C THYMUS CELLS ON THE ANTI-SRBC RESPONSE OF NUDE MICE[a]

GROUP	TREATMENT	No. OF ANIMALS	CELLS/SPLEEN	PFC/10^6 D	PFC/10^6 I	PFC/SPLEEN D	PFC/SPLEEN I	SERUM ANTIBODY HA	SERUM ANTIBODY HL
I	MDC + 2 thymuses	5	5.01×10^7	12	11	2092	1860	16	32
II	MDC + 2 thymuses + 5×10^7 T cells	4	3.94×10^7	39	29	5865	4195	40	180
III	5×10^7 T cells	4	4.48×10^7	9	5	950	555	13	60

a. Animals in groups I and II were implanted intraperitoneally with MDC containing 2 neonatal Balb/c thymus glands. 24 hours later animals in groups II and III were given 5×10^7 Balb/c thymus cells intraveneously. Two weeks later, these animals were challenged IP with 1×10^8 SRBC and assayed for a five day response.

controls (Table VII), they did respond better than animals in either group one or two, which received the MDC containing the thymus glands alone or the T-cells alone.

Homograft immunity and anti-SRBC response of nude mice and their littermates after the administration of polyanions

The results of attempts to reconstitute nude animals using the two polyanions PAA and dextran SO_4 are shown in Tables VII and VIII. In Table VII, animals were given 1.3 mg PAA I.P., alone or two hours prior to the administration of 1×10^8 SRBC. Five days after the administration of the antigen, the spleens of these animals were harvested for studies on humoral antibody levels. As can be seen, nude animals given PAA and SRBC did respond better than nudes given only SRBC, but this increased response is evident only when expressed per spleen and not when expressed per 10^6 spleen cells. Similarly, litter mate animals responded better to SRBC when given PAA than without PAA, but this increase is again evident only when expressed per spleen and not per 10^6 spleen cells. These findings are in agreement with those of Diamanstein (46 and 47) in normal NMRI/HAN mice. Humoral antibody studies reveal that both hemagglutinating and hemolytic antibody titers are increased when either nude mice of their litter mates are given SRBC and PAA as opposed to SRBC alone. That the PAA does not cause a

TABLE VII

ANTI SRBC RESPONSE OF NUDE AND LITTER MATE MICE GIVEN PAA AND OR SRBC[a]

	NO. ANIMALS	TREATMENT	PFC/10^6	PFC/SPLEEN	SERUM ANTIBODY HA	SERUM ANTIBODY HL	TOTAL NUCLEATED SPLEEN CELL COUNT
NUDES	13	PAA + SRBC	19	3655	318	340	1.59×10^8
	11	SRBC	18	1520	168	97	9.74×10^7
	6	PAA	2	113	23	7	3.60×10^7
	5		3	220	0	0	1.83×10^8
LITTER MATES	9	PAA + SRBC	386	152240	1564	2206	4.76×10^8
	14	SRBC	320	46129	1067	1587	1.34×10^8
	11	PAA	1	438	2	1	4.56×10^8
	12		3	177	0	0	1.19×10^8

a. On day 0, animals receiving PAA and SRBC were given 1.3 mg PAA 2 hours prior to the administration of 1×10^8 SRBC IP. On Day 5, all animals were bled for sera and their spleens were plaqued for specific PFC.

non specific increase in anti-SRBC PFC is evident in that neither nude animals nor their litter mates were able to produce high numbers of PFC or high titers of HA and HL antibodies.

Table VIII shows the results of similar experiments using the polyanion dextran SO_4. Animals receiving both dextran SO_4 and SRBC were given a total of 0.5 mg dextran SO_4 I.P. one half hour prior to the administration of 1×10^8 SRBC. Increased numbers of PFC were seen in both nudes and their litter mates when dextran SO_4 was given before antigen. Humoral antibody studies also revealed that the administration of dextran SO_4 would increase both the HA and HL titers of litter mate mice. Nude mice, however, gave an increased HL titer but a slightly depressed HA titer when given dextran SO_4 before SRBC. With the exception of the PFC/10^6 response of one nude mouse (see appendix G), all animals given dextran SO_4 alone gave very low anti-SRBC responses, indicating that the dextran SO_4 did not cause a nonspecific increase in the number of antibody producing cells.

The effects of the polynucleotides polyadenylic-polyuridylic acid on the anti-SRBC response of normal litter mate mice are given in Table IX. Mice treated with both SRBC and poly A:U were given 1×10^8 SRBC and 0.08 mg poly A:U simultaneously. Assay on day 5 revealed although animals receiving both poly A:U and SRBC did not produce as many PFC/10^6 spleen cells as did animals receiving only SRBC, they

TABLE VIII

ANTI SRBC RESPONSE OF NUDE AND LITTER MATE MICE GIVEN DEXTRAN SO_4 AND OR SRBC[a]

	NO. ANIMALS	TREATMENT	PFC/10^6	PFC/SPLEEN	SERUM ANTIBODY HA	HL	TOTAL NUCLEATED SPLEEN CELL COUNT
NUDES	8	Dextran SO_4 + SRBC	32	2780	118	225	1.07×10^8
	11	SRBC	18	1520	168	97	9.74×10^7
	6	Dextran SO_4	15	563	0	0	1.67×10^8
	5	Untreated	3	220	0	0	1.83×10^8
LMC	11	Dextran SO_4 + SRBC	403	96355	1408	1793	2.09×10^8
	14	SRBC	320	46129	1067	1587	1.34×10^8
	12	Dextran SO_4	4	117	5	8	
	12	Untreated	3	177	0	0	1.19×10^8

a. On day 0, animals receiving dextran SO_4 were given 0.5 mg IP ½ hour prior to the IP administration of 1×10^8 SRBC. On day 5, the animals were bled for sera and their spleens were plaqued for specific PFC.

did produce higher numbers of PFC when expressed per spleen. The number of indirect plaques is higher than the number of direct plaques in both the poly A:U plus SRBC and the SRBC only treated animals. Humoral antibody studies also reveal an increase in both the HA and the HL titers in the poly A:U treated mice. That the poly A:U did not produce a nonspecific increase in antibody forming cells is evidenced by the low response of mice given poly A:U alone.

Table X shows the effect of polyanions on the homograft rejection times of normal litter mate mice. The three polyanions, poly A:U, dextran SO_4 and PAA (0.08 mg, 0.5 mg, and 1.3 mg amounts respectively) were administered I.P. at 12 hours, 24 hours, 48 hours, and 72 hours after the grafting of 6 week old male CBA skin. Column 4 gives the mean graft survival times in days for each group. Animals treated with polyanions failed to reject their homografts in an accelerated fashion.

TABLE IX

ANTI SRBC RESPONSE OF LITTERMATE MICE GIVEN POLY A:U AND OR SRBC[a]

NO. ANIMALS	TREATMENT	PFC/10^6		PFC/SPLEEN		SERUM ANTIBODY		TOTAL NUCLEATED SPLEEN CELL COUNT
		D	I	D	I	HA	HL	
10	Poly A:U SRBC	108	194	80,858	118,990	1152	2432	1.67×10^8
9	Poly A:U Only	0	0	189	113	9	4	2.53×10^8
2	SRBC Only	206	528	62,500	108,250	960	1920	2.06×10^8
3	Untreated	0	0	340	113	0	0	2.09×10^8

a. On day 0, animals were given 0.08 mg poly A:U and or 1×10^8 SRBC IP. On day 5, the animals were bled for sera and their spleens were plaqued for specific PFC.

TABLE X

HOMOGRAFT IMMUNITY OF LITTERMATE MICE GIVEN POLY ANIONS[2]

GROUP	No. OF ANIMALS	TREATMENT	MEAN GRAFT SURVIVAL TIME
I	6	Poly A:U CBA Homograft	11.3 days
II	6	Dextran SO_4 CBA Homograft	11.7 days
III	6	Poly Acrylic Acid CBA Homograft	11.8 days
IV	6	No chemicals CBA Homograft	11.0 days

a. Animals were given 0.08 mg. poly A:U, 0.5 mg. dextran SO_4, or 1.3 mg PAA I.P. at 12 hours, 24 hours, 48 hours and 72 hours after the grafting of 6 week old male CBA skin.

DISCUSSION

That thymus-dependent immune reactions become operative in nude animals grafted with Balb/c thymus glands is evident from Table I. Thymus-dependent homograft immunity was established in the thymus grafted group but not in the sham operated group. These findings are in agreement with those of Wortis (64). Evidence for immunological memory in the system stems from the decreased rejection times associated with secondary homografts. Similarly, increased PFC, RFC and serum antibody titers specific for SRBC support the conclusion that thymus-dependent immune functions were operating. That these thymus-dependent immune responses were not equal to those of litter mates to nudes is evident from the faster homograft rejection times (Table X) and increased anti-SRBC responses of similarly immunized litter mates (Table VII). These responses may have been of greater magnitude if the thymus glands had been in place for a longer period of time before assay for immunological competence. In addition to the decreased immunological responses as compared to litter mates, though wasting syndromes associated with nude mice were delayed in the thymus-grafted group, the health and life span of the animals did not equal that of the litter mate animals.

The failure of CBA thymus glands (Table II) to reconstitute the anti-CBA homograft immunity in nude animals is in marked contrast to similar experiments in neonatally thymectomized mice where investigations

(77) have revealed that neonatally thymectomized mice grafted with allogeneic thymic tissue in adult life were able to later reject identical allogeneic skin grafts and indeed the original grafted thymus glands. That these thymus glands were functioning is evident from the increased serum antibody levels specific for SRBC when the animals were thymus-grafted, but these responses are much lower than the increases seen in nudes grafted with Balb/c thymus glands (Table I). One reason for the disparity in homograft data in the nude system grafted with allogeneic thymus glands and similar systems in neonatally thymectomized systems may be that in the neonatally thymectomized animal, prior to the removal of the thymus glands, some T cells may be released into the peripheral lymphoid system. When these animals are later grafted with an allogeneic thymus, a soluble THF analogous to that described earlier (33) may be released to activate those few clones of host lymphocytes, which then undergo blast transformation into mature immunocompetent cells capable of rejecting the allongeneic thymus and any histoincompatible skin grafts, In nude animals, which are congenitally athymic, these initial low numbers of thymus-derived lymphocytes are never seeded so the THF produced by grafted thymuses has an effect only on thymocytes seeded from the grafted thymus glands. Failure of nude precursoral cells that have migrated through the thymus and out into the peripheral lymphoid tissues to respond to the

allogeneic thymus glands and similar skin grafts probably result from an overwhelming tolerogenic effect on them as they pass through the antigenic environment of the grafted thymuses.

Failure to establish thymus-dependent immune responses to SRBC (Table III) and homografts (Table IV) in nude females which had given birth to wild type young is in contrast to Osoba's studies (36) involving a similar system in neonatally thymectomized mice. In Osoba's system the transplacental passage of some THF produced by the thymus glands of the developing young served to expand low numbers of thymus-derived cells which had been seeded before thymectomy. In nude animals, the lack of this peripheral pool of thymus-derived cells leaves the THF with no target cell in the mother so she is incapable of becoming immunocompetent with respect thymus-dependent immune responses. Alternatively, it is possible that in the nude mouse THF is not able to cross the placenta as easily as in other strains of mice used in neonatal thymectomy work.

A similar explanation may apply to the disparity of results obtained here with nude animals bearing MDC containing thymus glands (Table V) and results obtained earlier (33 and 34) with neonatally thymectomized mice bearing MDC containing thymus glands. Presumably, the passage of some THF from the glands through the millipore membrane served to activate and expand the few thymus-derived cells which were

seeded before neonatal thymectomy. In nude animals, this may not be possible because the thymus-derived cells do not exist.

Experiments designed to confirm that THF may indeed act upon low numbers of immature thymus-derived cells present in peripheral lymphoid tissues generally did not lead to decisive conclusions (Table VI). Though nude mice receiving both low numbers of thymus cells and MDC containing two thymus glands did respond slightly better than nudes which had received either thymus cells or MDC containing two thymus glands alone, the increase was small and due largely to the abnormally high response of one animal in the group (see appendix D). One reason for the apparent inability of low numbers of Balb/c thymus cells to expand and become functional under the influence of THF in the nude system may be that the cells become subject to allogeneic inhibition before they are able to begin expansion. Though our nude colony was established in a Balb/c background, the two lines are still not congenic so the thymus cell inocula may have been inactivated through allogeneic inhibition.

Results involving the use of the synthetic polynucleotide, poly A:U, and the two polyanions, dextran SO_4 and PAA, in nudes and their litter mates confirm the conclusions drawn by other investigators using these chemicals in other strains of mice. Cone and Johnson (44) have concluded that poly A:U may effect the immune response by increasing

the number of available thymus-derived cells. Data presented in Table IX do not directly confirm this but they do provide evidence for an increase in both the number of PFC/spleen and in humoral antibody levels. The failure of poly A:U treated litter mates given CBA homografts to reject skin faster than controls (Table X) suggests either that the cells responsible for skin rejection are not responsive to the chemical or that the natural homograft rejection process is more efficient than the artificial process.

Diamantstein et al. (47) have suggested that polyanions such as PAA and dextran SO_4 may effect the immune response by replacing or mimicing the effect of some natural product (possibly of anionic character also) released by thymus-derived cells during their interaction with antigen. This natural product and polyanions in general may act to confer antibody synthesizing ability to bone marrow derived lymphocytes. Alternatively, polyanions may effect the immune response by concentrating the antigen in such a manner as to allow for a more effective presentation to bone marrow-derived lymphocytes in the absence of thymus-derived cells.

Failure to decrease homograft rejection times in litter mate mice given CBA/j skin grafts and then polyanions (Table X) may imply that either thymus-derived cells responsible for destruction of homografts are not responsive to these chemicals and the natural

thymus-derived cell free product, whose action these chemicals mimic, or that these chemicals are not able to expand this killer cell population as well as the thymus-derived cell population responsible for antigen recognition.

SUMMARY

Earlier reports that nudes are deficient in their ability to respond to thymus-dependent antigens such as SRBC and to homografts were confirmed. Attempts to establish immune competence in these animals met with varying degrees of success. The grafting of whole Balb/c thymus organs to the axial region did allow nudes to produce higher numbers of PFC, RFC and higher levels of specific antibodys when challenged with SRBC. Also they were able to reject homografts in both primary and secondary set fashion. The use of CBA/j thymus glands, however, did not allow nudes to reject CBA skin homografts. Use of soluble thymic factors in nudes generally did not increase their responses to SRBC nor did it allow them to reject homografts. Attempts to establish immune competence using chemicals did increase the number of PFC/spleen and the levels of serum antibodys in both nudes and their litter mates, though nude responses were still much smaller than those of their litter mates. Implications of these findings to the normal immune system were discussed.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Askonas, B. A., and R. G. White. 1956. Sites of antibody production in the guinea pig. The relation between invitro synthesis of anti ova-albumin and globulin and distribution of antibody containing plasma cells. British Journal of Experimental Pathology. 37:61.

2. Harris, T. N., J. Rhoads, and J. Stokes, Jr. 1948. A study of the role of the thymus and spleen in the formation of antibody in the rabbit. J. Immunology. 58:27.

3. Maclean, L. D., S. J. Zak, R. L. Varco, and R. A. Good. 1957. The role of the thymus in antibody production: An experimental study of the immune response in thymectomized rabbits. Transplantation Bulletin. 4:21

4. Good, R. A. 1954. Agammaglobulnemia: A provocative experiment of nature. Bulletin University. Minnesota Hospital and Minnesota Medical Foundation. 26:1.

5. Gafni, J., D. Michaili, and H. Heller. 1960. Idiopathic acquired agammaglobulinemia associated with thymoma: Report of two cases and Review of the literature. New England Journal of Medicine. 263:536.

6. Glick, G., T. S. Chang, and R. G. Japp. 1956. The bursa of fabricus and antibody production. Poultry Science. 35:224.

7. Muller, A. P., H. R. Wolfe and R. K. Meyer. 1960. Precipitin production in chickens. XXI. Antibody production in bursectomized chickens and in chickens injected with 19-nortestosterone on the fifth day of incubation. J. Immunology. 85:172.

8. Miller, J. F. A. P. 1961. Immunological functions of the thymus. Lancet. 2:748.

9. Martinez, C., J. Kersey, B. W. Papermaster and R. A. Good. 1962. Skin homograft survival in thymectomized mice. Proc. Soc. Exp. Biol. and Med. 109:193.

10. Metcalf, D. 1964. The thymus and Lymphopoiesis. In: The Thymus in Immunobiology, edited by R. A. Good and A. E. Gabriesen. New York: Hoeber-Harper, page 150.

11. Andreasen, E., and J. Ottesen. 1945. Studies on the lymphocyte production. Investigations on the nucleic acid turnover in lymphoid organs. Acta Physiologica. Scandanavia. 10:258.

12. Murray, R. G., and P. A. Woods. 1964. Studies on the fate of lymphocytes. III. The migration and metamorphosis of in situ labelled thymic lymphocytes. Anatomical Records. 150:113.

13. McGregor, D. D., and J. L. Gowans. 1963. The antibody response of rats depleted of lymphocytes by chronic drainage from the thoracic duct. J. Experimental Medicine. 117:303.

14. Dougherty, T. F. 1952. Effect of hormones on lymphatic tissue. Physiological Reviews. 32:379.

15. Jutila, J. W. 1969. Wasting disease induced with cortisol acetate. III. Immunologic studies. J. of Immunology. 102:963.

16. Mitchel, G. F., and J. F. A. P. Miller. 1968. The cellular basis of the immunological defects in thymectomized mice. I. The thoracic duct lymphocyte output of unanaesthetized mice thymectomized at birth. Nature. 214:992.

17. Parrott, D. M. V., M. A. B. DeSousa, and J. East. 1966. Thymus-dependent areas in the lymphoid organs of neonatally thymectomized mice. J. of Experimental Medicine. 123:191.

18. Miller, J. F. A. P. and D. Osoba. 1967. Current concepts of the Immunological function of the thymus. Physiological Reviews. 47:437.

19. Good, R. A., A. P. Dalmasso, C. Martinez, O. K. Archer, J. C. Pierce and B. W. Papermaster. 1962. The role of the thymus in development of immunologic capacity in rabbits and mice. J. Experimental Medicine. 116:773.

20. Dalmasso, A. P., C. Martinez, and R. A. Good. 1962. Failure of spleen cells from neonatally thymectomized mice to induce graft versus host reactions. Proc. Soc. Expt. Biol. and Med. 110:205.

21. Rieke, W. O. 1966. Lymphocytes from thymectomized rats: Immunologic, proliferative and metabolic properties. Science. 152:535.

22. Fisher, E. R. and B. Fisher. 1963. Role of the thymus in skin and tumor transplantation in the rat. Transplantation. 14:546.

23. Martinez, C., A. P. Dalmasso, and R. A. Good. 1962. Acceptance of tumor homografts by thymectomized mice. Nature. 194:1289

24. Osoba, D. and N. Auersperg. 1966. Growth of a human carcinoma of the cervix in mice thymectomized at birth. J. National Cancer Institute. 36:523.

25. Fichtelius, K. E., G. Laurell and L. Philipsson. 1961. The influence of thymectomy on antibody formation. Acta Path. Microbiol. Scand. 51:81.

26. Cross, A. N., E. Leuchars, and J. F. A. P. Miller. 1964. Studies on the recovery of the immune response in irradiated mice thymectomized in adult life. J. Experimental Medicine. 119:837.

27. Miller, J. F. A. P. 1964. Effect of thymic abalation and replacement. In: The Thymus in Immunobiology, edited by R. A. Good and A. E. Gabrielsen. New York. Hoeber-Harper, page 436.

28. 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

29. Harris, J. E. and C. E. Ford. 1964. Cellular traffic of the thymus: Experiments with chromosomal markers. Evidence that the thymus plays an instructional part. Nature. 201:884.

30. 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 leathally irradiated mice. Proc. Roy. Soc. (London), Series B. 165:78.

31. Harris, J. E., C. E. Ford, D. W. H. Barnes and E. P. Evans. 1964. Cellular traffic of the thymus: Experiments with chromosome markers. Evidence from parabiosis for an efferent stream of cells. Nature. 201:886.

32. Dalmasso, A. P., C. Martinez, K. Sjodin, and R. A. Good. 1963. Studies on the role of the thymus in immunobiology. Reconstitution of immunologic capcacity in mice thymectomized at birth. J. Experimental Medicine. 118:1089.

33. Levey, R. H., N. Trainin and L. W. Law. 1963. Evidence for function of thymic tissue in diffusion chambers implanted in neonatally thymectomized mice. J. National Cancer Institute. 31:199.

34. Osoba, D., and J. F. A. P. Miller. 1963. Evidence for a humoral thymus factor responsible for the maturation of immunologic faculty. Nature. 199:653.

35. Osoba, D. 1965. Immune reactivity in mice thymectomized soon after birth: normal response after pregnancy. Science. 147:295.

36. Metcalf, D. 1958. The thymus lymphocytosis stimulating factor. Ann. N. Y. Acad. Sciences. 73:113.

37. DeSomer, P., P. J. Denyn and R. Leyten. 1963. Activity of a non-cellular calf thymus extract in normal and thymectomized mice. Life Science. 2:810.

38. Trainin, N. and M. Linker. 1967. Restoration of immunologic reactivity of thymectomized mice by calf thymus extracts. Cancer Research. 27:309.

39. Small, M. and N. Trainin. 1967. Increase in antibody forming cells of neonatally thymectomized mice receiving calf thymus extract. Nature (London). 216:377.

40. Fishman, M. and F. L. Addler. 1963. Antibody formation initiated in-vitro. II. Antibody synthesis in X-irradiated recepients of diffusion chambers containing nucleic acids derrived from macrophages incubated with antigen. J. Experimental Medicine. 117:595.

41. Fishman, M., and F. L. Adler. 1963. Antibody formation initiated in vitro. II. Antibody synthesis in X-irradiated recipients of diffusion chambers containing nucleic acids derrived from macrophages incubated with antigen. J. of Experimental Medicine. 117:595.

42. Brown, W. and E. P. Cohen. 1971. On the role of nucleic acids in antibody formation. In: Regulation of the Antibody Response, edited by B. Cinader and C. C. Thomas. Springfield, page 349.

43. Friedman, H. P., A. B. Stavitsky, and J. M. Soloman, 1965. Induction in-vitro of antibodies to phage T2: Antigens in the RNA extract employed.

44. Cone, R. E., and A. G. Johnson. 1971. Regulation of the immune response by synthetic polynucleotides. J. Experimantal Medicine. 113:665.

45. Diamantstein, T., B. Wagner, I. Beyse, M. V. Odenwald and G. Schulz. 1971. Stimulation of humoral antibody formation by poly anions. I. The effect of polyacrylic acid on the primary immune response in mice immunized with sheep red blood cells. Eur. J. of Immunology. 1:335.

46. Diamantstien, T., B. Wagner, I. Beyse, M. V. Odenwald and G. Schulz. 1971. Stimulation of humoral antibody formation by polyanions. II. The influence of sulfate esters of polymers on the immune response of mice. Eur J. of Immunology. 1:340.

47. Diamantstein, T., B. Wagner, J. L'Age-Stehr, I. Beyse, M. V. Odenwald, and G. Schulz. 1971. Stimulation of humoral antibody formation by polyanions. III. Restoration of the immune response to sheep red blood cells by polyanions in thymectomized and leathally irradiated mice protected with bone marrow cells. Eur. J. of Immunology. 1:302.

48. Humphrey, J. H., D. M. V. Parrot, and J. East. 1964. Studies on globulin and antibody production in mice thymectomized at birth. Immunology. 7:419.

49. Doenhoff, M. J., A. J. S. Davies, E. Leuchers, and V. Wallis. 1970. The thymus and circulating lymphocytes of mice. Proc. Roy. Soc. (London) B. 176:69.

50. Flanagan, S. P. 1966. Nude, a new hairless gene with pleiotropic effects in the mouse. Genetic Research (Cambridge). 8:295.

51. Pantelouris, E. N. Absence of thymus in a mouse mutant. Nature 217:370.

52. Raff, M. C. and H. H. Wortis. 1970. Thymus dependence of bearing cells in the lymphoid tissues of mice. Immunology. 18:931.

53. Kindred, B. 1971. Immunological unresponsiveness of genetically thymusless (nude) mice. Eur. J. of Immunology. 1:59.

54. Wortis, H. H. 1971. Immunological responses of "nude" mice. Clinical Experimental Immunology. 8:305.

55. Pantelouris, E. M., and P. A. Flisch. 1972. Responses of athymic ("nude") mice to sheep red blood cells. Eur. J. of Immunology. 2:236.

56. Reed, N. D., and J. W. Jutila. 1972. Immune response of congenitally thymusless mice to heterologous erythrocytes. Proc. Soc. Exp. Biol. and Med. 139:1234.

57. Manning, J. K., N. D. Reed, and J. W. Jutila. 1972. Antibody response to Escherichia coli lipopolysaccharide and type III pneumococcal polysaccharide by congenitally thymusless (nude) mice. J. Immunology. 108:1470

58. Rygaard, J. 1969. Immunobiology of the mouse mutant "nude." Acta Path. Microbiol Scand. 77:761.

59. Pantelouris, E. M. 1971. Observations on the immunobiology of "nude" mice. Immunology. 20:247.

60. Rygaard, J. and C. O. Povlsen, 1969. Heterotransplantation of a human malignant tumor to "nude" mice. Acta.Path. Microbiol. Scand. 77:758.

61. Povlsen, C. O. and J. Rygaard. 1971. Heterotransplantation of Human adenocarcinomas of the colon and rectum to the mouse mutant nude. A study of nine consecutive transplants. Acta. Path. Microbial. Scand. 79:159.

62. Giovanella, B. C., S. O. Yim, J. S. Stehlin, and L. J. Williams. 1972. Development of invasive tumors in the "nude" mouse after injection of cultured human melanoma cells. J. Nat. Cancer Institute. 48:1531.

63. deSousa, M. A. B., D. M. U. Parrott and E. M. Pantelouris. 1969. The lymphoid tissues in mice with congenital aplasia of the thymus. Clinical Experimental Immunology. 4:637.

64. Wortis, H. H., S. Hehlsen, and J. J. Owen. 1971. Abnormal development of the thymus in nude mice. J. Experimental Medicine 134:681.

65. Pantelouris, E. M. 1970. Observations on the Immunobiology of "nude" mice. Immunology. 20:247.

66. Kindred, B. 1971. Antibody response in genetically thymusless mice injected with normal thymus cells. J. Immunology. 107:1291.

67. Kindred, B. and E. Weiler. 1971. The response to SRBC by nude mice injected with lymphoid cells other than thymus cells. J. Immunology. 109:382.

68. McPherson, C. W. 1963. Laboratory Animal Care. 13:737.

69. Bartlett, G. L., and R. T. Prehn. 1969. An improved design for general purpose diffusion chambers. Transplantation. 7:

70. Hunter, J. T. 1968. Master's thesis, Montana State University.

71. Mishell, R. I., and R. W. Dutton. 1967. Immunization of dissociated spleen cell cultures from normal mice. J. Experimental Medicine. 126:423.

72. Boizzi, G., C. Stiffel, and D. Moutom. 1967. in Immunity, Cancer and Chemotherapy. (E. Mihich, ed.). Academic Press, New York. p. 103.

73. Addler, F. L. 1965. Studies on mouse antibodies. J. Immunology. 95:26.

74. Campbell, D. H., J. S. Garvey, N. E. Cremer, and D. H. Sussdorf. 1970. Methods in Immunology (2nd ed.) W. A. Benjamin Inc., New York.

75. Billingham, R. E., and W. K. Silvers. 1961. Transplantation of Tissues and Cells. The Wistar Institute Press, Philadelphia.

76. Pilgram, J. and G. DeOme. 1955. Intraperitoneal pentobarbitol anesthesia in mice. Experimental Medicine and Surgery. 13:401.

77. Miller, J. F. A. P. 1962. Role of the Thymus in transplantation immunity. Ann. N. Y. Acad. Sci. 99:340.

APPENDIXES

APPENDIX A

HOMOGRAFT RESPONSE AND ANTI-SRBC RESPONSE OF NUDES GRAFTED WITH BALB/c THYMUS GLANDS OR SHAM GLAFTED NUDES[a]

EXP. #	MOUSE #	1° HOMO-GRAFT	2° HOMO-GRAFT	PFC/10^6 D	PFC/10^6 I	PFC/SPLEEN D	PFC/SPLEEN I	SERUM HA	ANTIBODY HL
8	Nude 1	15	7					320	40
	3							320	160
	5							1280	320
	6	15	8					640	1280
	8							160	80
20	Nude A1	13	13	165	284	12200	21000	640	2560
	A3	18	9	274	233	36510	31000	1280	1280
	A5	18	13	583	770	34500	46000	1280	5120
	A6	18	9	95	102	12100	13000	2560	2560
	A7	28	11	180	349	9800	19000	640	2560
	A10	28	13	66	72	6800	7500	640	640
	B1	15	13	0	1	40	200	0	0
	B4		13	16	19	1560	1840	0	40
	B9	24							
	B10	9							
28	Nude 3	18		93	52	10600	5900		
	4	20		0	0	100	120		
	6	23		625	634	27000	27400		
8	Nude 4							20	0
	9							40	0
	10	45	15					40	20
	11							80	80
20	Nude A2		23	17	13	2000	1540	40	320
	A4	54	23	10	8	1060	860	40	80
	A8	54	23	10	15	1120	1640	20	0
	A11	24							
	B5	50	23	13	14	1320	1360	40	40
	B2	50	23					40	40
	B3	20							
	B7	30							
	B11	29							
	B12	28							
28	Nude 13	26		167	107	7680	4900		
	14	26		6	4	1600	1180		

a. These data are summarized in Table I

APPENDIX B

ANTI-SRBC RESPONSE OF VIRGIN NUDES, VIRGIN LITTER MATE CONTROLS AND NUDE FEMALES WHICH HAD DELIVERED WILD TYPE YOUNG[a]

EXP. #	MOUSE #	PFC/10^6 D	PFC/10^6 I	PFC/SPLEEN D	PFC/SPLEEN I	SERUM ANTIBODY HA	SERUM ANTIBODY HL
1	81	5	3	550	320	0	0
1	86	4	3	700	540	0	80
1	47	10	4	850	380	80	160
2	2	21	7	1804	633	80	160
2	3	19	4	836	165	320	160
2	3	32	18	660	386	320	320
2	52	28	31	4680	5530	640	320
3	217	3		320		20	10
3	207	3		160		0	0
4	263	33		7300		320	320
4	264	35		6200		160	80
1	80	8	9	1020	1180	160	320
1	125	10	5	540	300	20	80
2	55	11	9	1722	1495	80	160
2	56	38	8	910	187	40	160
2	51	45	37	3250	2695	320	320
3	1	6		1040		320	0
3	2	0		0		0	0
4	240	14		1400		80	80
4	250	12		1080		320	160
1	1	213	233	20480	22400	640	5120
1	2	142	288	19840	40320	1280	5120
2	1	182	542	19250	57200	1280	5120
2	2	155	517	20900	69850	1280	5120
2	3	294	590	41112	82225	1280	5120
3	1	27		5200		1280	2560
3	2	53		6800		640	1280
4	1	292		45000		2560	2560

a. Data are summarized in Table III

APPENDIX C

HOMOGRAFT RESPONSE AND ANTI-SRBC RESPONSE OF NUDES GRAFTED WITH EITHER MDC CONTAINING NEONATAL THYMUS GLANDS OR EMPTY MDS[a]

EXP. #	MOUSE #	PFC/10^6 D	PFC/10^6 I	PFC/SPLEEN D	PFC/SPLEEN I	SERUM ANTIBODY HA	SERUM ANTIBODY HL	HOMO-GRAFT REJ.(days)
13	Nude 1					10	0	
	3					320	320	
	4					80	80	
27	Nude 1			740	480			28
	3			3560	3900			28
	8			3160	3300			28
13	Nude 5					40	80	
	6					40	40	
27	Nude 9			4060	1860			28
	13			1800	1080			28
	14			180	120			28
37	Nude 7							24
	8	27	23	5240	4440	20	40	24
	9							19
	10	10	18	420	800	10	10	24

a. Data are summarized in Table V.

APPENDIX D

THE EFFECT OF THYMIC HUMORAL FACTOR AND OR BALB/c THYMUS CELLS ON THE ANTI-SRBC RESPONSE OF NUDE MICE[a]

GROUP	MOUSE #	PFC/10^6 D	PFC/10^6 I	PFC/SPLEEN D	PFC/SPLEEN I	SERUM ANTIBODY HA	SERUM ANTIBODY HL
	3	4	8	800	1640	0	0
	4	1	1	280	160	0	0
I	7	4	3	1400	1160	0	0
	14	48	43	7700	6100	80	160
	15	3	2	280	240	0	0
II	2	12	5	1140	560	0	0
	9	113	105	16000	14800	160	640
	12	8	1	1340	200	0	0
	13	23	6	4980	1220	40	80
III	1	1	1	240	320	0	0
	5	28	17	2280	1400	40	160
	8	6	2	1060	360	10	80
	11	2	1	220	140	0	0
IV[b]	16	729	871	149000	178000	2560	2560

a. Data are summarized in Table VI

b. Group IV consisted of a single littermate control mouse, immunized and plaqued along with the experimental mice as a control on the plaqueing technique itself.

APPENDIX E

ANTI-SRBC RESPONSE OF NUDES AND LITTER CONTROLS GIVEN PAA AND SRBC[a]

EXP. #	MOUSE #		PFC/10^6 D	PFC/10^6 I	PFC/SPLEEN D	PFC/SPLEEN I	SERUM ANTIBODY HA	SERUM ANTIBODY HL	CELLS/ SPLEEN[b]
14	Nude	2	1	1	160	440	320	160	79.4
		4	10	6	5600	3440	320	640	144
15	Nude	1	10		1640		640	160	162
		2	37		5720		640	320	154
		3	13		2520		320	160	178
		4	14		1080		320	80	138
		5	26		8200		640	320	312
		6	41		6360		320	320	149
16	Nude	2	21		3040		10	320	145
		6	9		2000		80	320	234
		7	40		6360		160	640	160
		8	25		3560		40	640	148
18	Nude	1	5		1280				65.2
14	LMC	1	195	TNTC	56000	TNTC	1280	2560	71.8
		2	200	TNTC	77000	TNTC	640	1280	96.5
15	LMC	1	122		133000		2560	5120	1080
17	LMC	3	272		134000		2560	2560	492
		5	159		201000		1280	2560	1270
		7	492		313000		2560	2560	636
30	LMC	1	442	652	224500	339000	1280	2560	520
		10	1054	2003	178000	339000	1280	2560	169
		13	577	1019	132500	234000	1280	640	230
		17	380	1118	73400	224000			193

a. These data are summarized in Table VII.

b. times 10^6

APPENDIX F

ANTI-SRBC RESPONSE OF NUDES AND LITTER MATE CONTROLS GIVEN PAA ONLY[a]

EXP. #	MOUSE #		PFC/10^6 D	PFC/10^6 I	PFC/SPLEEN D	PFC/SPLEEN I	SERUM ANTIBODY HA	SERUM ANTIBODY HL	CELLS/ SPLEEN[b]
14	Nude	5	1	0	120	0	40	0	51.4
15	Nude	9	1		80		80	40	129
16	Nude	5	1		120		20	0	121
		10	4		160		0	0	36
		11	0		0		0	0	62.8
30	Nude	24	4	12	200	680	0	0	55.6
14	LMC	3	0	1	40	560	0	0	97.1
15	LMC	3	0		120		10	0	267
16	LMC	1	1		200		0	0	238
		2	0		80		10	0	428
		3	0		80		0	0	346
		4	1		200		0	0	269
17	LMC	8	2		1400		0	0	804
		11	1		880		0	0	604
		12	1		680		0	0	484
30	LMC	15	2	2	1140	880	0	10	576
		18	0	0	0	0	0	0	904

a. These data are summarized in Table VII

b. times 10^6

APPENDIX G

ANTI-SRBC RESPONSE OF NUDES AND LITTER MATE CONTROLS GIVEN DEXTRAN SO_4 AND SRBC[a]

EXP. #	MOUSE #		PFC/10^6 D	PFC/10^6 I	PFC/SPLEEN D	PFC/SPLEEN I	SERUM ANTIBODY HA	SERUM ANTIBODY HL	CELLS/ SPLEEN[b]
4	Nude	1	2	7	520	200	0	320	36
		2	21	16	3760	2920	80	320	184
		3	6	5	1200	1000	20	80	202
22	Nude	1	20	9	1800	840	160	80	92.4
		2	38	48	5400	6800	320	640	143
24	Nude	1	34	25	2160	1560	0	0	63.6
		4	90	32	2120	760	40	40	23.5
		5	46	41	5240	4640	320	320	114
4	LMC	1	555	725	92000	120000			166
		2	780	TNTC	235000	TNTC			294
22	LMC	1	173	809	19200	90000	2560	1280	111
		2	145	237	31800	52000	1280	1280	220
24	LMC	1	1110	TNTC	147000	TNTC	640	2560	132
30	LMC	3	241	634	28000	73500	640	0	116
		5	282	946	32000	107500	1280	10	114
		8	324	1001	43500	134500	1280	2560	134
		11	345	1105	111500	357100	1280	2560	329
		14	415	662	225400	360000	1280	2560	544
		20	68	68	94500	94500	1280	2560	140

a. These data are summarized in Table VIII

b. times 10^6

APPENDIX H

ANTI-SRBC RESPONSE OF NUDES AND LITTER MATE CONTROLS GIVEN DEXTRAN SO_4 ONLY[a]

EXP. #	MOUSE	#	PFC/10^6 D	PFC/10^6 I	PFC/SPLEEN D	PFC/SPLEEN I	SERUM ANTIBODY HA	SERUM ANTIBODY HL	CELLS/SPLEEN[b]
4	Nude	7	0	0	40	40	0	0	395
		8	2	1	280	80	0	0	126
22	Nude	3	0	1	0	240	0	0	196
		4	1	2	80	240	0	0	147
24	Nude	2	8	5	920	440	0	0	114
		6	80	12	2060	320	0	0	26
4	LMC	4	1	1	240	200			230
22	LMC	3	1	1	80	80	0	0	79.2
		4	1	0	100	20	0	0	87.6
24	LMC	4	37	5	280	40	0	0	76.0
		6	0	0	100	40	0	0	548
30	LMC	2	0	0	20	40	0	0	248
		6	0	0	0	0	0	0	168
		9	0	0	40	0	0	0	189
		12	0	0	40	0	10	10	692
		16	0	0	80	60	40	80	322
		19	0	0	160	100	0	0	648
		21	2	0	260	0	0	0	148

a. These data are summarized in Table VIII

b. times 10^6

APPENDIX I

ANTI-SRBC RESPONSE OF NUDES AND LITTER MATE CONTROLS GIVEN SRBC ONLY[a]

EXP. #	MOUSE #		PFC/10^6 D	PFC/10^6 I	PFC/SPLEEN D	PFC/SPLEEN I	SERUM ANTIBODY HA	SERUM ANTIBODY HL	CELLS/ SPLEEN[b]
4	Nude	4	3	1	360	200	10	20	137
		5	13	7	2680	1400	80	160	209
		6	5	5	1280	1160	20	40	256
14	Nude	7	7	10	400	560	160	0	14.4
15	Nude	7	3		240		640	320	78.0
		8	34		2440		160	160	72.4
18	Nude	3	8		240				29.5
		4	1		40				37.3
22	Nude	5	7	15	640	1400	320	80	91.6
		6	21	21	1600	1600	40	10	76.0
24	Nude	3	97	25	6800	1720	80	80	70.0
4	LMC	3	0	0	0	0	0	0	175
14	LMC	5	288	421	21200	31000	640	1280	18.4
15	LMC	2	329		32400		1280	2560	98.4
17	LMC	1	469		63000		2560	2560	134
		2	347		52000		1280	640	150
		4	206		30000		1280	1280	146
		10	64		11600		320	320	181
18	LMC	3	563		54000				96.0
		5	476		71000				149
22	LMC	5	178	414	12000	28000	640	160	67.6
		6	133	150	41000	46420	1280	1280	77.2
24	LMC	5	547	1229	76600	172000	1280	2560	140
26	LMC	19	314	558	85000	151000	640	2560	270
		22	304	497	40000	65500	1280	1280	132
30	LMC	22	257	409	56000	89000	320	2560	218

a. These data are summarized in Tables VII and VIII
b. times 10^6

APPENDIX J

HOMOGRAFT IMMUNITY OF LITTERMATE CONTROLS GIVEN POLYANIONS[a]

MOUSE #	# DAYS TAKEN TO REJECT CBA SKIN	POLY ANION USED	AVERAGE FOR THE GROUP
A1	10	Poly A:U	
A2	11	Poly A:U	
A3	9	Poly A:U	
A4	12	Poly A:U	11.3 days
A5	14	Poly A:U	
A6	12	Poly A:U	
B7	12	Dextran SO_4	
B8	12	Dextran SO_4	
B9	15	Dextran SO_4	
B10	11	Dextran SO_4	11.7 days
B11	11	Dextran SO_4	
B12	9	Dextran SO_4	
C13	12	PAA	
C14	11	PAA	
C15	12	PAA	
C16	9	PAA	11.8 days
C17	15	PAA	
C18	12	PAA	
D19	11	None	
D20	11	None	
D21	9	None	
D22	10	None	11.0 days
D23	13	None	
D24	12	None	

a. These data are summarized in Table X