Brief Definitive Report

THE ADOPTIVE TRANSFER OF PREGNANCY-INDUCED UNRESPONSIVENESS TO MALE SKIN GRAFTS WITH THYMUS-DEPENDENT CELLS*

By R. N. SMITH and A. E. POWELL

(From the Departments of Reproductive Biology and Surgery, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106)

The genetically alien fetus survives far beyond the time required to reject grafts genetically identical to that fetus. Two strains of animals which mutually reject grafts will nevertheless have normal pregnancies and hybrid offspring without apparent fetal or neonatal immunological disease which could be caused by a maternal response to fetal transplantation antigens. Many theories which could account for this paradox have been reviewed recently (1, 2).

The most compelling and provocative of these theories has evolved from data provided by in vitro assays of cell-mediated immunity which have shown that as a consequence of an allogeneic pregnancy maternal lymphocytes respond to paternal but not to third-party antigens (3, 4). These responses are blocked specifically by autologous maternal serum suggesting that maternal humoral factors might inhibit in vivo the potential pathological activity of maternal lymphocytes for fetal tissues which bear transplantation antigens (3, 4). Pregnancy thus becomes a state of immunological enhancement.

The development of in vivo models to determine how pregnancy influences the maternal response to paternal antigens has not met with uniform success. Reports (1, 2, 5, 6) suggest that the female, as a consequence of pregnancy, delays rejecting grafts histocompatible with the allogeneic fetus, but there is also evidence suggesting that pregnancy does not alter the rate of rejection of grafts (1, 2, 7). The discrepancies are probably methodological since hosts of different parity, strains of different histocompatibility, and a variety of test grafts were employed.

A readily available model to study the influence of pregnancy on the way in which the female responds to transplantation antigens is the pregnancy-induced unresponsiveness to the male-specific transplantation antigen of the mouse, H-Y (6). Virgin female mice of certain strains reject male skin grafts due to the H-Y antigen. After many syngeneic pregnancies females show delayed rejection of male skin grafts and often become tolerant of such grafts (6). The pregnancy-induced hyporesponsiveness to male skin grafts is specific for the male antigen since female grafts, histoincompatible at minor antigens, (H-4), do not enjoy prolonged survival on hosts which otherwise would show delayed rejection of syngeneic male skin grafts (8).

* Supported by NIH-NICHD-HD-00024, Ford Foundation grant 670-135 A, NCI contract N01-CB-43990, and Sigma XI of Cleveland.

In the present study lymphoid tissues and sera from females made unresponsive to the H-Y antigen by pregnancy were transferred to virgin recipients which were immediately test grafted with male skin. It was found that thymus-dependent lymphocytes but not sera from tolerant donors transferred unresponsiveness.

Materials and Methods

Animals. C57BL/10Sn (B10) male and female mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. Females were either virgin or multiparous six or more times by B10 males.

Operative Procedure. Female mice were grafted with male ear skin (9), and the grafts were scored as rejected if obvious necrosis had occurred or if gentle scraping with a fingernail removed the test grafts.

Cell Suspensions. Tissues were pressed through a wire screen into minimal essential medium (MEM), supplemented with 10% fetal calf serum (FCS) and 10 mM Hepes buffer (all from Grand Island Biological Co., Grand Island, N. Y.), pelleted at 400 *g*, washed once, and the desired number of viable cells injected intraperitoneally (i.p.) into virgin B10 female recipients. All preparative procedures with cells were done at 4°C.

Separation of Splenic T and B Cells. Mouse splenic cells were separated into T- and B-lymphocyte subpopulations according to the method of Julius et al. (10). The criterion used for analysis of splenic lymphocyte subsets was the relative cytotoxicity of antibody and complement (C) to the Thy 1.2 antigen of mice. AKR anti-C3H thymocyte ascitic fluid (Litton Bionetics, Kensington, Md.) killed 35–40% of B10 splenic cells, greater than 90% of B10 thymocytes, and less than 5% of bone marrow cells. Anti-theta treatment of B10 splenic cells reduced the mitogen-induced proliferation to phytohemagglutinin by 75%, to concanavalin A by 90%, and to lipopolysaccharide by less than 10%. The nonadherent fractions of splenic cells passed over nylon wool columns were called splenic T lymphocytes if greater than 90% of the cells were killed with the anti-theta and C. This analysis was done for every adoptive transfer experiment. Cells adherent to the nylon wool were removed from the column by repeated gentle compression of the syringe plunger, and the resulting cell suspension contained 5–10% theta-bearing cells which were removed with anti-theta antibodies and C. Nylon-adherent splenic cells remaining after treatment with anti-theta and C were called B cells but also contained other cells. Controls were treated with C alone.

Adoptive and Passive Transfers. Cells were injected i.p. into virgin B10 females immediately after grafting with male ear skin. The elapsed time between grafting and inoculation of test cells was 1–4 h. 0.5 ml of sera pooled from multiparous tolerant donors was injected on days −3, −1, 0, +1, and +3, into virgin females which were grafted with male skin on day 0.

Results

To accumulate tolerant donors, retired female breeders pregnant six or more times were grafted with male skin, and the results are summarized in Table I. 60% of multiparous females did not reject male skin grafts, a distribution similar to that reported by Billingham et al. (6) for a comparable degree of parity.

Lymphoid cells from females, virgin or multiparous with long standing male skin grafts (80–120 days), were transferred i.p. to virgin female recipients grafted with male skin. The data summarized in Table II and Figs. 1 and 2 reflect an extensive series of adoptive transfer experiments in which tissues from various donors were injected into virgin test recipients to determine if sera or lymphoid cells from multiparous females tolerant of male skin could influence the rate at which virgin females rejected their grafts.

Tolerance was transferred to a majority of recipients with unfractionated spleen cells (A), thymocytes (D), or splenic T cells (G) from multiparous females tolerant of male skin. The transfer of fewer than 3×10^7 splenic cells/g recipient

TABLE I
Survival of Male Skin Grafts on B10 Females

n	Recipient	No. of surviving grafts		
		35 days	60 days	+100 days
50	Virgin	10	1	1
75	Multiparous	60	55	45

Recipients were B10 females, virgin or pregnant six or more times by B10 males, and were grafted with B10 male skin. Median survival time for virgins was 26.5 days, and for multiparous hosts the median survival time was +100 days.

TABLE II
Adoptive Transfer of Tolerance to Male Skin Grafts

Group	n	Tissue	Donor	Cells transferred $\times 10^7$/g recipient	Number of surviving grafts		
					35 days	60 days	+100 days
A	15	Spleen cells	Multiparous	3	15	12	8
B	10	Spleen cells	Multiparous	1.7-2	7	2	0
C	20	Spleen cells	Virgin	3	5	0	0
D	15	Thymocytes	Multiparous	3	14	11	9
E	20	Thymocytes	Virgin	3	6	0	0
F	10	Bone marrow	Multiparous	3	3	0	0
G	15	Spleen T cells	Multiparous	3	15	12	10
H	10	Spleen T cells	Virgin	3	3	0	0
I	15	Spleen B cells	Multiparous	3	2	0	0
J	15	Tolerant sera	Multiparous	–	4	0	0
K	10	Spleen cells	Sensitized	3	0	0	0

All recipients were virgin B10 females grafted with male ear skin. Cells were transferred i.p. 0.5-ml aliquots of pooled sera from tolerant donors were given on days −3, −1, 0, +1, and +3. Animals were grafted on day 0.

resulted only in the prolonged survival of male skin grafts (B). Splenic cells (C), thymocytes (E), or splenic T cells (H) from virgin females lacked any activity, and recipients rejected their grafts at a normal rate. Bone marrow cells from tolerant donors did not transfer any discernible hyporesponsiveness (F), and splenic B cells from multiparous tolerant donors appeared to lack activity (I) and could not transfer tolerance unlike T cells from the same splenic population. Sera from tolerant donors after passive transfer did not effect the rate at which virgin females rejected their male skin grafts (J). Splenic cells from donors which had rejected one male skin graft adoptively transferred accelerated graft rejection to virgin recipients (K).

Discussion

Tolerance to the H-Y antigen induced by multiparity was transferred to virgin recipients with thymus-dependent lymphocytes but not with B cells. Sera from donors made tolerant of male skin grafts by multiparity were also unable to prolong the survival of male skin grafts in virgin females confirming earlier work (8, 11) in which sera from multiparous donors were unable to transfer any

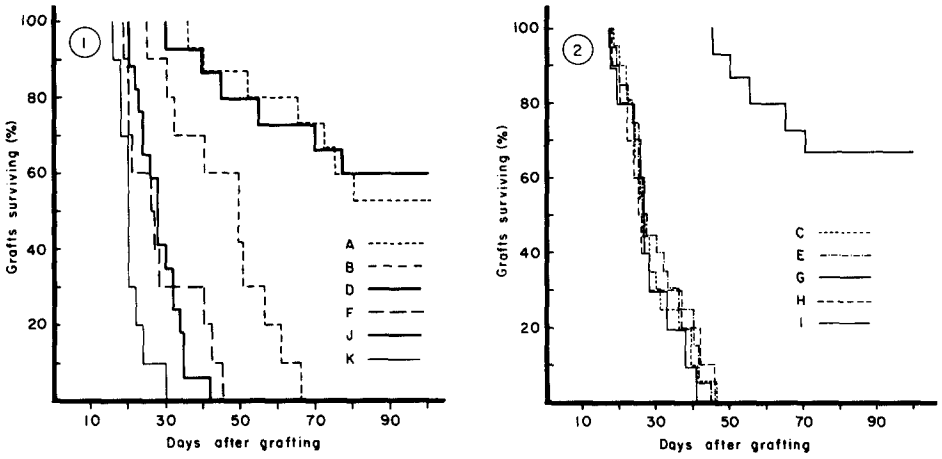

FIG. 1. The adoptive transfer of pregnancy-induced unresponsiveness. Groups are the same as those found in Table II.

FIG. 2. The adoptive transfer of pregnancy-induced unresponsiveness by splenic T cells. Groups are the same as those found in Table II.

degree of hyporesponsiveness. It is unlikely, therefore, that pregnancy-induced unresponsiveness to the H-Y antigen is a state of immunological enhancement and wholly attributable to serum-borne factors.

It is also unlikely that other models of tolerance to skin allografts are examples of immunological enhancement since sera from tolerant donors do not enhance grafts passively (12, 13). Kilshaw et al. (12) and Brent et al. (13) using different models of unresponsiveness to allografts were unable to transfer any degree of hyporesponsiveness with sera from tolerant donors.

Although sera from donors made tolerant by multiparity were unable to transfer tolerance, thymus-dependent lymphocytes from the same donors could transfer tolerance. Thus, the adoptive transfer of pregnancy-induced unresponsiveness described in Table II and Figs. 1 and 2 bears a striking resemblance to the report of Kilshaw et al. (14) who transferred adult-induced allograft tolerance in mice with T lymphocytes and to the report of Dorsch and Roser (15) who transferred neonatally-induced tolerance of allografts with thoracic duct T lymphocytes.

The model of neonatally-induced tolerance is undoubtedly similar to pregnancy-induced unresponsiveness in one other way. Hosts made unresponsive to allografts by neonatal inoculation of lymphoid cells are chimeric as adults (16), and pregnancy causes at least transient chimerism since fetal cells are found in the mother (1, 2). But male cells exist at undetectable levels in females tolerant of the H-Y antigen by multiparity (6) and could not transfer unresponsiveness to adult animals by themselves even if they constituted 5% of the transferred population (6, 15). In addition if chimeric male cells alone transferred tolerance, then lymphoid cells other than T cells should transfer tolerance, a result not observed in Table II. Because too few chimeric cells could have been transferred in the present experiments to induce tolerance in the recipient mice (6), a population of maternal T cells also must be required and may in fact account entirely for the transfer of the unresponsive state.

Nevertheless, chimerism is necessary to maintain neonatally-induced tolerance (17), and the degree of chimerism is related directly to the number of normal or immune cells required to break tolerance (18). Thus viable chimeric cells are not mere bystanders but play an active part maintaining the unresponsive state. Therefore, because fetal cells (or at least fetal antigen) are found in the mother as a consequence of pregnancy, the influence of fetal antigen on the induction and maintenance of pregnancy-induced unresponsiveness must be considered to have an important function.

The data in Table II and Figs. 1 and 2 confirm other reports (14, 15) that hosts unresponsive to transplantation antigens maintain thymus-dependent lymphocytes capable of suppressing the rejection of grafts, and that sera from tolerant animals are alone incapable of transferring tolerance (8, 11–13). This phenomenon is inexplicable solely in terms of the clonal deletion of antigen-reactive cells or the humoral abrogation of cell-mediated immunity. In addition, because the adoptively transferred unresponsiveness to the H-Y antigen was induced by pregnancy, the maternal response to paternal antigens during pregnancy may be actively limited at least in part by maternal thymus-dependent lymphocytes.

Summary

A majority of C57BL/10Sn females pregnant six or more times by syngeneic males do not reject male skin grafts. The pregnancy induced tolerance of male skin grafts was transferred adoptively to virgin recipients by thymus-dependent cells from multiparous tolerant donors.

The authors thank Doctors J. C. Howard, A. Stavitsky, and J. W. Streilein for helpful discussions during the course of this work and during the preparation of this manuscript.

Received for publication 22 April 1977.

References

1. Beer, A. E., and R. E. Billingham. 1971. Immunobiology of mammalian reproduction. *Adv. Immunol.* 14:1.
2. Edwards, R. B., and R. R. A. Coombs. 1975. Immunological interactions between mother and fetus. *In* Clinical Aspects of immunology. P. G. H. Gell, R. R. A. Coombs, and P. J. Lachman, editors. Blackwell Scientific Publications Ltd., Oxford. 561.
3. Pence, H., W. M. Petty, and R. E. Rocklin. 1975. Suppression of maternal responsiveness to paternal antigens by maternal plasma. *J. Immunol.* 114:525.
4. Hellstrom, K. E., I. Hellstrom, and J. Brawn. 1969. Abrogation of cellular immunity to antigenically foreign embryonic cells by a serum factor. *Nature (Lond.).* 224:914.
5. Breyere, E. J., and M. K. Barrett. 1960. Prolonged survival of skin homografts in parous female mice. *J. Natl. Cancer Inst.* 25:1405.
6. Billingham, R. E., W. K. Silvers, and D. B. Wilson. 1965. A second study on the H-Y transplantation antigen in mice. *Proc. R. Soc. Lond. B. Biol. Sci.* 163:61.
7. Medawar, P. B., and E. M. Sparrow. 1956. The effects of adrenocortical hormones, adrenocorticotrophic hormone, and pregnancy on skin transplantation immunity in mice. *J. Endocrinol.* 14:240.
8. Jeekel, J. 1973. The behavior of male skin grafts in isogenic postpartum female mice. *Transplantation (Baltimore).* 16:570.
9. Barker, C., and R. E. Billingham. 1973. Skeletal muscle as a privileged site for orthotopic skin allografts. *J. Exp. Med.* 138:289.

10. Julius, M. A., E. Simpson, and L. A. Herzenberg. 1971. Rapid method for the isolation of functional thymus-derived murine lymphocytes. *Eur. J. Immunol.* 3:645.
11. Kaliss, N., and M. K. Dagg. 1964. Immune response engendered in mice by multiparity. *Transplantation (Baltimore).* 2:416.
12. Kilshaw, P. J., L. Brent, and A. V. Thomas. 1974. Specific unresponsiveness to skin allografts in mice. II. The mechanism of unresponsiveness induced by tissue extracts and antilymphocyte serum. *Transplantation (Baltimore).* 17:57.
13. Brent, L., C. Brooks, N. Nubling, and A. V. Thomas. 1972. Attempts to demonstrate an *in vivo* role for serum blocking factors in tolerant mice. *Transplantation (Baltimore).* 14:382.
14. Kilshaw, P. J., L. Brent, and M. Pinto. 1975. Suppressor T cells in mice made unresponsive to skin allografts. *Nature (Lond.).* 255:489.
15. Dorsch, S., and B. Roser. 1975. T cells mediate transplantation tolerance. *Nature (Lond.).* 258:233.
16. Billingham, R. E., and L. Brent. 1959. Quantitative studies on tissue transplantation immunity. IV. Induction of tolerance in newborn mice and studies on the phenomenon of runt disease. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 242:439.
17. Lubaroff, D. M., and W. K. Silvers. 1973. The importance of chimerism in maintaining tolerance of skin allografts in mice. *J. Immunol.* 111:65.
18. Silvers, W. K., and R. E. Billingham. 1969. Influence of the Ag-B locus on reactivity to skin homografts and tolerance responsiveness in rats. *Transplantation (Baltimore).* 8:167.