

Influence of Breast Feeding on Subsequent Reactivity to a Related Renal Allograft¹

WILLIAM E. KOIS, M.D.,² DARRELL A. CAMPBELL, JR., M.D.,³ MARC I. LORBER, M.D.,
JAMES C. SWEETON, B.S., M.S., AND DONALD C. DAFOE, M.D.

Department of Surgery, University of Michigan Hospitals, Ann Arbor, Michigan 48109

Submitted for publication November 10, 1983

In a previous report the hypothesis that exposure of the neonate to maternal allogeneic cells via the process of breast feeding would result in hyporesponsiveness to a subsequent maternal donor-related renal transplant was examined. Support for this hypothesis was obtained after correlating results of maternal donor-related renal transplantation with the breast feeding status of the transplant recipient. In the present report this observation has been expanded upon and it was asked if a history of breast feeding was associated with improved results in a different patient population (HLA semi-identical sibling donors). Breast-fed patients showed dramatic improvements in graft function rates compared to non-breast-fed counterparts at all intervals studied ($P \leq 0.001$). Because a history of breast feeding correlated with improved results after sibling donor as well as maternal donor transplantation, it was concluded that the breast feeding effect is not entirely specific for maternal antigens. These observations underscore the importance of breast feeding as a variable in clinical-related renal transplantation.

INTRODUCTION

Experimental observations in rat systems have demonstrated that hyporesponsiveness to maternal antigens may be induced in offspring by the process of breast feeding [3]. The mechanism involved in this interesting phenomenon is not well understood, but appears to involve at least partial absorption of maternal breast milk alloantigen by the neonatal GI tract. We recently described what appears to be a parallel phenomenon in clinical transplantation [5, 6]. Recipients of maternal donor-related renal transplants fared far better if they had been breast fed by their donor during infancy. This suggested that very early exposure to the alloantigenic cells known to be present in human breast milk resulted in subsequent hyporesponsiveness to these antigens, possibly in the same way that experi-

mental animals have been made hyporesponsive to antigen by neonatal exposure [4].

In the present study we have expanded upon this observation and asked if a history of breast feeding was associated with improved results in a different patient population. Those recipients having received allografts from HLA semi-identical siblings were chosen for study because, like the maternal donor recipients, they shared one haplotype with their donor. However, in these cases recipients were not challenged with a maternal kidney. Our results indicate that breast feeding is also associated with improved results after subsequent sibling donor HLA semi-identical transplantation. These studies suggest a nonspecific mechanism for hyporesponsiveness induced by this variable.

PATIENTS AND METHODS

During the years 1964-1981 59 sibling-sibling related renal transplants were performed in which donor and recipient shared one haplotype. Mailed inquiries and telephone conversations were made to obtain information

¹ Presented at the Annual Meeting of the Association for Academic Surgery, Syracuse, New York, November 2-5, 1983.

² Present address: Department of Rehabilitation Medicine, Tufts University Medical Center, Boston, Mass. 02155.

³ To whom requests for reprints should be addressed.

about maternal breast feeding of the transplant recipient and donor. In 42 cases reliable information was obtained regarding whether recipient and donor had been breast fed during infancy. In 39/42 cases either both donor and recipient had been breast fed or both donor and recipient had not been breast fed. Of the remaining three patient combinations two recipients had been breast fed while the donor had not and in one case the situation was reversed. In an attempt to limit the number of variables under consideration the latter three patients were not evaluated as part of this study. Breast fed (BF) in this communication thus refers to patient pairs in which both recipient and donor were breast fed, while non breast fed (NBF) refers to patient pairs in which neither recipient nor donor had been breast fed. Mothers were invariably able to recall whether or not a particular individual had been breast fed but were often vague as to the length of time breast feeding had been done. This information, while potentially important, was not considered in the interpretation of results.

Of 39 patient pairs evaluated, 24 had been breast fed while 15 had not been breast fed. These groups were then compared with regard to certain variables known to influence results in transplantation (Table 1). No significant differences were noted between groups with

TABLE 1
COMPARISON OF GROUPS

	Breast fed	Not breast fed
Number of patients	24	15
Recipient age	29.8	24.7
Donor age	29.5	25.3
Male/female	12/12	7/8
Preoperative blood transfusion	19/24 (79%)	13/15 (87%)
Native nephrectomy	9/24	0/15
Duration of dialysis (months)	12.8	9.8
ATG	4	2
Diabetes	0	2
Splenectomy	1	0

regard to recipient age, donor age, male/female ratio, percentage of patients receiving preoperative blood transfusions, duration of preoperative dialysis, or patients receiving anti-thymocyte globulin. There were two diabetic patients in the population studied and both were in the NBF group. Only one patient had undergone splenectomy. A significant difference between groups was noted for the number of patients having undergone native nephrectomy; 9/24 patients in the BF group had had such a procedure while 0/15 of the NBF patients had undergone nephrectomy.

By definition, patients included in this study shared one haplotype with their donor, breast fed or not. Mixed lymphocyte culture data was obtained only in the most recently transplanted patients (since 1977) and these data were not considered in this study. No patient included in this series underwent donor-specific transfusion.

Transplantation of the allograft was performed in standard fashion, using an end-end internal iliac artery to renal artery arterial anastomosis in most cases and an external ureteroneocystostomy. Immunosuppression was accomplished with Prednisone (1.5 mg/kg initially) and Imuran (2.5 mg/kg initially). After 1970, rejection episodes were routinely treated with high-dose "pulse" therapy consisting of 30 mg/kg of methylprednisolone.

Statistical analysis of graft survival data was done using Breslow's generalized Kruskal-Wallis analysis.

RESULTS

Patient survival. Of the 39 patients involved in this study one death occurred in the first post transplant year, resulting in a 1-year patient survival of 97%. This patient, in the BF group, died of bacterial sepsis with a normal serum creatinine.

Graft function. Breast-fed recipients of HLA semi-identical sibling donor transplants experienced markedly improved rates of graft function when compared to their NBF counterparts (Fig. 1). Statistically significant improvements in graft function were noted for

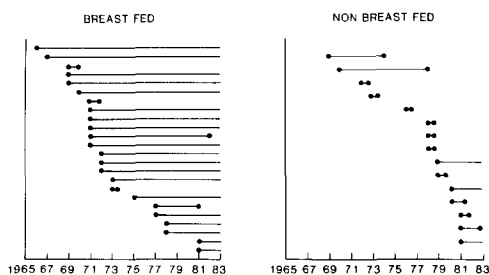


FIG. 1. Graft function in BF versus NBF recipients of sibling donor HLA semi-identical related renal transplants. The X axis denotes the year in which the transplant was performed. A closed circle at the end of the line indicates that the transplant rejected.

BF recipients at all intervals ($P \leq 0.0015$). Not only did BF recipients show remarkably good results (cumulative proportion surviving at 9 years = 79%) but NBF recipients were found to have done very poorly (cumulative proportion surviving at 9 years = 15%) (Table 2).

Rejection episodes in the first post-transplant year. Six of 24 patients (25%) having a BF history failed to have a discernable rejection episode in the first post-transplant year. The comparable figure in the NBF group was 1/15 (6%). This difference did not obtain statistical significance (Table 2).

Number of antirejection pulses in the first post-transplant year. Breast-fed and NBF groups were evaluated with respect to the number of "pulse" treatments used to treat rejection within the first postoperative year. Although a trend toward more pulses in the

NBF group was seen, the differences did not achieve statistical significance. Many patients in the NBF group completely rejected their grafts within a few months of transplantation; following loss of the graft they obviously would not receive additional "pulses." For this reason we also calculated the data as number of pulses per month of function (Table 2). NBF patients received significantly more pulses per month of function within the first post-transplant year than did BF counterparts ($P \leq 0.01$).

DISCUSSION

We have previously reported that recipients of maternal donor-related renal transplants experienced improved results if they had been breast fed during infancy [5, 6]. Because of these findings we advanced the hypothesis that exposure of the neonate to maternal breast milk resulted in subsequent hyporesponsiveness to maternal antigens. Breast feeding was not associated with improved results if the patient was subsequently transplanted with a paternal donor kidney, a finding which suggested a certain degree of specificity to the phenomenon. The purpose of the present study was to expand upon these observations using a different group of related renal transplant recipients. We chose sibling donor HLA semi-identical patient pairs for study since these patients, as in the previous study, had HLA semi-identical relationships with their donor. However, because the transplanted kidney was not of maternal origin, results in

TABLE 2

GRAFT FUNCTION AND CLINICAL COURSE OF BREAST FED VERSUS NON-BREAST-FED PATIENT GROUPS

	Breast fed (24)	Not breast fed (15)	P
Graft function			
1 year (%)	88	47	≤ 0.001
5 years (%)	79	30	≤ 0.001
9 years (%)	79	15	≤ 0.001
No rejection in first year	6/24 (25%)	1/15 (6%)	NS
Pulses/patient in first year	3.5 ^a	5.1	NS
Number of pulses/month function	0.29 ^a	0.74	≤ 0.01

^a Data on 21 patients. Three patients were excluded because they underwent a rejection episode prior to the advent of "pulse" therapy with methylprednisolone.

this group would provide another test for the specificity of our original observation. A breast feeding history in the sibling-sibling combination was associated with dramatically improved results. Because of these findings it seems necessary to conclude that the breast feeding effect described in our first communication, and further documented in this study, is not entirely specific for maternal antigens.

The magnitude of the differences noted between BF and NBF groups in this study was not anticipated. Not only did the BF recipients do extremely well (9-year graft function rate of 79%), but the NBF recipients did extremely poorly. Poor results in the NBF group occurred despite the fact that these patients received significantly more immunosuppression than did the BF group. It might justifiably be argued that the numbers of patients in each group was relatively small. The small numbers reflect the fact that we defined our groups very narrowly, i.e., all patients studied were recipients of sibling donor HLA semi-identical grafts. The only covariable noted which could have biased results was the observation that significantly more BF than NBF patients had undergone native nephrectomy. We do not feel it is likely that this variable could account for the great differences noted between groups. No other covariables differed significantly; particularly important was the observation that the percentage of patients having undergone preoperative blood transfusion was almost identical.

Whether or not it is important that the donor be breast fed is not known, since we had very few instances in which the recipient was not breast fed and the donor was, or vice versa. With regard to this point, however, it is interesting to note that transfusion history of cadaveric donors has been found to be an important variable influencing results in cadaveric transplantation [8]. The dramatic differences noted between BF and NBF groups in this study may be related to the fact that in this study the BF history of the donor was also considered.

A number of experimental observations, taken together, support the hypothesis that

breast feeding has a favorable influence on the course of a subsequent renal transplant. First, it is known that antigen administered during the neonatal period often results in immune unresponsiveness to that antigen [4]. Second, the oral route of administration of antigen itself often favors tolerance induction [1, 2, 11, 12, 15]. Finally, it is known that there exists abundant maternal alloantigen in the breast milk of most species [7, 10, 14]. A critical question, however, involves the extent to which a neonate would be exposed to maternal alloantigen when the route of administration is via the intestinal lumen. However, a variety of recent studies have demonstrated that the neonate is systemically exposed to maternal cells after ingestion of breast milk [3, 13, 16]. In one particularly interesting report, radio-labeled allogeneic lymph node cells were fed to newborn rats and later detected within the gastric wall as well as in draining mesenteric lymph nodes, indicating that absorption of the cells had taken place [17].

On the basis of the available experimental data, and the clinical correlates described in this and previous reports [5, 6], it seems likely that the process of breast feeding is a variable which influences result in organ transplantation. Additional clinical studies with larger numbers of patients are needed to substantiate our observations. In particular it will be interesting to note whether a breast feeding history influences results in cadaveric transplantation. While the mechanism accounting for improved results following breast feeding is not yet defined, there appears to be a similarity to the well documented observation that administration of blood transfusions enhances the results of subsequent renal transplantation. Our studies raise the possibility that, with regard to subsequent organ transplantation, the process of breast feeding represents a first and very important transfusion.

REFERENCES

1. Andre, L., Bazin, H., and Biozzi, G. IgA antibody response to intragastric immunization in high and low immune responder lines of mice. *Eur. J. Immunol.* 7: 246, 1977.

2. Andre, L., Heremons, J. F., Vaerman, J. P., *et al.* A mechanism for the induction of immunological tolerance by antigen feeding: Antigen-antibody complexes. *J. Exp. Med.* **142**: 1509, 1975.
3. Beer, A. E., Billingham, R. E., and Head, J. R. Natural transplantation of leukocytes during suckling. *Transpl. Proc.* **7**: 399, 1975.
4. Billingham, R. E., Brent, L., and Medewar, P. B. Actively acquired tolerance of foreign cells. *Nature (London)* **172**: 603, 1953.
5. Campbell, D. A., Jr., Lorber, M. I., Sweeton, J. C., *et al.* Maternal donor related renal transplants: Influence of breast feeding on reactivity to the allograft. *Transpl. Proc.* **15**(1): 906, 1983.
6. Campbell, D. A., Jr., Lorber, M. I., Sweeton, J. C., *et al.* Breast feeding and maternal donor related renal allografts: The original donor specific transfusion? Transplantation, in press.
7. Diaz-Joranen, E. P., and Williams, R. C. T and B lymphocytes in human colostrum. *Immunol. Immunopathol.* **3**: 248, 1974.
8. Frisk, B., Beiglin, E., and Brynger, H. *Transplantation* **35**: 352, 1983.
9. Hanson, D. G., Vaz, N. B., Maia, L. C. S., *et al.* Inhibition of specific immune responses by feeding protein antigens. *Int. Arch. Allergy Appl. Immunol.* **55**: 526, 1977.
10. Head, J. E., and Beer, A. E. In B. Latson (Ed.), *Lactation: A Comprehensive Treatise*. New York: Academic Press, 1978. Pp. 337.
11. Kagnoff, M. F. Functional characteristics of Peyer's patch lymphoid cells. IV. Effect of antigen feeding on the frequency of antigen-specific B cells. *J. Immunol.* **118**: 922, 1977.
12. Mattingly, J. A., and Waksman, B. H. Immunologic suppression after oral administration of antigen. I. Specific suppressor cells formed in rat Peyer's patches after oral administration of sheep erythrocytes and their systemic migration. *J. Immunol.* **121**: 1878, 1978.
13. Ogra, S. S., Weintraub, and Ogra, P. L. Immunologic aspects of human colostrum and milk. III. Fate and absorption of cellular and soluble components in the gastrointestinal tract of the newborn. *J. Immunol.* **119**: 245, 1977.
14. Parmely, N. J., Beer, A. E., and Billingham, R. E. *In vitro* studies on the T lymphocyte population of human milk. *J. Exp. Med.* **144**: 358, 1976.
15. Rigan, J., and Kind, L. S. Suppressor T cells for IgE and IgG in Peyer's patches of mice made tolerant by the oral administration of Ovalbumin. *J. Immunol.* **120**: 861, 1978.
16. Seelig, L. L., and Billingham, R. E. Concerning the natural transplantation of maternal lymphocytes via milk. *Transpl. Proc.* **13**(1): 1245, 1981.
17. Seelig, L. L., and Head, J. R. Evidence that maternally derived lymphocytes delivered via the milk transit the neonatal digestive tract epithelium, submitted.