

# Essential Fatty Acid Depletion of Renal Allografts and Prevention of Rejection

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A central hypothesis in transplantation biology is that resident leukocytes expressing class II histocompatibility antigens may determine the immunogenicity of an organ. By means of a novel method to deplete the kidney of resident leukocytes, essential fatty acid deficiency (EFAD), this hypothesis was tested in an intact, vascular organ. Kidneys subjected to EFAD and thus depleted of resident Ia-positive macrophages survived and functioned when transplanted across a major histocompatibility antigen barrier in the absence of immunosuppression of the recipient. Control allografts were rejected promptly. Allografts from donors subjected to EFAD normalized their lipid composition and were repopulated with host macrophages by 5 days. Administration of Ia-positive cells at the time of transplantation established that the resident leukocyte depletion induced by EFAD was responsible for the protective effect. These observations may provide insights into the mechanisms underlying tissue immunogenicity and the population of normal tissues with resident leukocytes.

THE ROLE OF CLASS II MAJOR HISTOCOMPATIBILITY complex (MHC) antigens in initiating allogeneic sensitization is well established (1). In an extension of Snell's hypothesis (2), passenger or resident leukocytes expressing class II MHC antigens have been postulated to be responsible for the immunogenic potential of organ allografts. Support for this hypothesis has been provided by the observations that rejection of rodent pancreatic islet and thyroid allografts can be prevented by subjecting the tissue to *in vitro* treatments that damage or remove passenger leukocytes, including culture in low temperature, exposure to ultraviolet irradiation, culture in

high O<sub>2</sub> tension, or exposure to antiserum directed against the Ia antigens of the donor (3, 4).

We previously characterized a resident macrophage population in the renal glomerulus of the rat that expresses Ia antigens and can serve as allogeneic stimulator cells in mixed lymphocyte cultures (5). Other investigators have established the presence of resident macrophages in the interstitium and glomeruli in mouse (6) and human kidneys (7). More recently, we observed that placement of rats on an essential fatty acid-deficient (EFAD) diet results in a marked depletion of Ia-positive macrophages from both the glomerulus and the interstitium (8). These findings provided the impetus to test whether this depletion might alter the immunogenicity of a renal allograft.

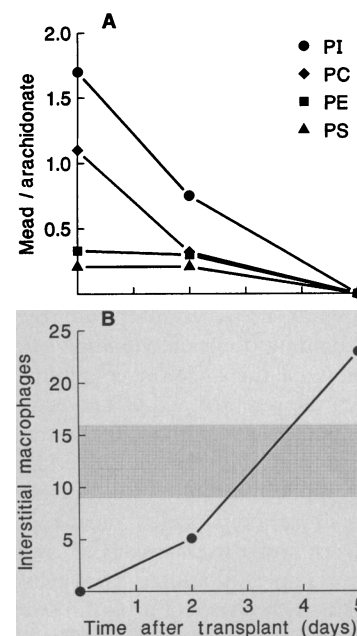
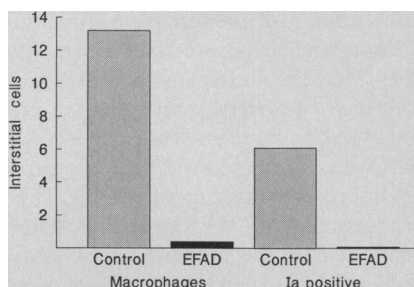
Lewis rats were placed on an EFAD diet for a minimum of 2 months before kidney donation. Hepatic and renal phospholipids showed the characteristic changes of the deficiency state (Table 1). All phospholipids manifested a depletion of arachidonate and an accumulation of 20:3(n-9), or Mead

acid, the fatty acid that accumulates in the EFAD state (9). Phosphatidylcholine and phosphatidylinositol were typically the most significantly affected phospholipids. These lipid changes were associated with nearly complete elimination of interstitial macrophages and Ia-positive cells from the kidney (Fig. 1).

In order to determine whether the depletion of resident macrophages diminished the allogeneic potential of the kidney, we subsequently transplanted kidneys from Lewis (RT1l) rats, fed either control or EFAD diets, to Buffalo strain rats (RT1b) differing at the MHC locus. The recipients were maintained on a normal diet and received no immunosuppressive therapy before or after the renal allograft. All kidneys transplanted from control Lewis rats on a normal diet to Buffalo recipients showed severe, irreversible cellular rejection by day 5, as judged by histological criteria (Table 2). In contrast, none of the EFAD donor kidneys showed any evidence of cellular infiltrate for up to 4 weeks after transplantation (Table 2). Sixteen recipients of EFAD allografts had their remaining native kidney removed at 2

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**Fig. 1.** Renal interstitial macrophage content. Renal cortex was dissociated into individual interstitial cells by a modification of a previously described method (8). Dissected renal cortex from saline-perfused kidneys was minced through a 250- $\mu$ m screen and subjected to mild enzymatic digestion consisting of collagenase type II (500  $\mu$ g/ml) (Worthington Biochemicals, Freehold, New Jersey), soybean trypsin inhibitor (1 mg/ml) (Sigma Chemical, St. Louis, Missouri), and DNAase (0.1  $\mu$ g/ml) (Sigma) in Hanks balanced salt solution for 30 min at 23°C. This procedure results in a single-cell suspension of tubular epithelial and interstitial cells along with intact glomeruli (8). The suspensions were then labeled for macrophage and Ia antigens (8). Positively labeled cells in the interstitial population were counted on a per volume basis and normalized for the weight of the dissected sieved cortex before digestion (expressed as 10<sup>5</sup> cells per gram of cortex). The bars reflect the average results of two separate experiments. Each experiment used a pool of cortex from two animals.



**Fig. 2.** Lipid and macrophage repletion of EFAD allografts. Tissue samples from EFAD allografts were obtained on days 0, 2, and 5. (A) EFAD allograft lipid analysis. The lipids from tissue samples from allografts were extracted, and the Mead acid to arachidonate ratio was determined by gas chromatographic analysis as detailed in the legend to Table 1. (B) EFAD allograft macrophage content. The macrophage content of the interstitium of EFAD allografts was determined in parallel with the lipid analysis. Details of macrophage quantification are provided in the legend to Fig. 1. The stippled area represents the range of normal values measured in rats on a standard diet. Abbreviations are explained in the legend to Table 1.

weeks. In six, the allografts were removed at 4 weeks and showed no evidence of cellular infiltrate. Allograft function was adequate to maintain survival, with a mean serum creatinine of  $1.6 \pm 0.2$  mg/dl at 2 weeks,  $1.5 \pm 0.2$  mg/dl at 4 weeks, and  $1.3 \pm 0.05$  mg/dl at 6 weeks after nephrectomy. The remaining group of ten Buffalo rat recipients of EFAD Lewis kidneys have survived over 6 months after nephrectomy of the native kidney.

Because the recipients were fed a control diet, partial normalization of the fatty acid composition of the EFAD allografts occurred at 2 days after transplantation and was essentially complete by day 5 (Fig. 2A). Over this same time period, the content of interstitial macrophages returned to a level slightly above normal (Fig. 2B). These cells expressed the class I MHC antigens of the

Buffalo recipient and appeared to represent repopulation of the interstitium with host leukocytes. The kinetics of lipid repletion and macrophage repopulation of the allograft appeared to correlate.

In subsequent experiments, Lewis Ia-positive splenic leukocytes were injected intraperitoneally into the Buffalo recipients at the time of the EFAD kidney transplant. All allografts in this category showed histological evidence of severe rejection on day 5 (Table 2). Splenocytes from EFAD or control Lewis rats were equally effective in inducing rejection of the kidney allograft. Thus the EFAD state did not protect the allograft against the effector phase of immune sensitization; rather, it prevented the induction of immune sensitization.

The observation that nonimmune cells, such as endothelial (10) and epithelial cells

(11) [including renal tubular cells (12)], can express Ia antigens either spontaneously or in response to immune stimuli has raised the possibility that intrinsic parenchymal cells may be capable of initiating allogeneic sensitization of recipients. Our experiments cannot assess relative contributions of marrow-delivered cells versus parenchymal cells in immune sensitization. They do suggest, however, that depletion of macrophages is sufficient to confer an immunologic neutrality that can be overridden by the provision of leukocytes at another site at the time of transplantation.

We have observed interstitial depletion of macrophages induced by the EFAD state in kidneys of Lewis, Buffalo, and Sprague-Dawley rats and also in pancreatic islets and heart in the Lewis rat. The mechanism by which the EFAD state depletes tissue of resident macrophages is unknown. One explanation may be a relative depletion of arachidonate metabolites. Glomeruli from EFAD animals synthesize markedly less prostaglandin  $E_2$  and thromboxane  $A_2$  in response to angiotensin II (8) and are severely limited in their capacity to synthesize leukotriene  $B_4$  (13). Alternatively, Mead acid, present only in the EFAD state, can be metabolized to a variety of eicosanoids of unknown biological significance (14). These could conceivably interrupt leukocyte traffic through the renal interstitium. Resolution of these questions should provide insights into transplantation biology and also into the interactions between organs and immune cells that regulate the population of tissues with resident leukocytes.

**Table 1.** Donor hepatic and renal phospholipid fatty acid composition. Rats were fed a fat-free diet or a standard laboratory diet (Purina Test Diets, Richmond, IN) for 8 weeks or longer. Liver and kidney samples from donor animals were removed at the time of donation for fatty acid analysis. Hepatic and renal phospholipids were extracted and separated by two-dimensional-thin-layer chromatography; the fatty acids within each phospholipid were then transmethyated and the resultant fatty acid methyl esters were characterized by gas chromatography as described (15). Results are expressed as percentage of total fatty acids or mol% (all points in triplicate, standard errors <10% of the mean). With EFAD, tissue 20:4(n-6) fell and 20:3(n-9) appeared. The 20:3(n-9) to 20:4(n-6) ratio in hepatic phospholipids exceeded 0.4, which is the biochemical definition of EFAD (16). Renal lipids, in contrast, exhibited more modest changes, as described (15). Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; and PS, phosphatidylserine.

Phospholipids	20:3(n-9)/20:4(n-6) (calculated ratio)			
	Hepatic phospholipid composition		Renal phospholipid composition	
	Control	EFAD	Control	EFAD
PC	0.0/33.0 (0.0)	16.6/4.5 (3.7)	0.0/26.5 (0.0)	10.5/9.5 (1.1)
PE	0.0/28.7 (0.0)	19.0/15.3 (1.2)	0.0/53.9 (0.0)	10.8/33.3 (0.3)
PI	0.0/48.4 (0.0)	39.0/2.9 (13.4)	0.0/37.9 (0.0)	23.6/13.9 (1.7)
PS	0.0/23.4 (0.0)	11.2/15.4 (0.7)	0.0/33.1 (0.0)	6.7/31.9 (0.2)

**Table 2.** Rejection of control and EFAD renal allografts. Kidneys were harvested from Lewis rat donors (both EFAD and normal animals) after thorough perfusion with saline containing papaverine (0.5 mg/ml). Papaverine addition was necessary to effect complete washout of circulating leukocytes. Buffalo rats on a normal diet were used as the recipients. The left kidney of the recipient was surgically removed, and the Lewis allograft was transplanted to the recipient as described (17). The native right kidney was left in situ. A small percentage of EFAD and control grafts failed as a result of ischemia or hydronephrosis (17% versus 10% for EFAD and control grafts, respectively) and were not included in the analysis. After 2, 5, or 14 days the allografts were removed to provide tissue for histologic analysis. In one group, the remaining native kidney was removed at day 14, and the allografts were allowed to function until day 28 and then removed for analysis. Rejection was defined by the presence of interstitial nephritis with periglomerular, perivascular, and peritubular infiltrates of mononuclear cells. Kidneys were defined as not rejecting when there was no cellular infiltrate and a normal parenchyma was observed. No intermediate abnormal histology was observed. In several experiments with EFAD grafts, recipients were also given an intraperitoneal inoculum of  $10^8$  Lewis spleen cells in concert with the allograft. In three animals EFAD spleen cells were given, and in two experiments control spleen cells were used. No significant difference between control and EFAD animals with respect to the percentage (approximately 15%) of spleen cells expressing Ia was seen. ND, not done.

Transplants	Rejected/total			
	2 days	5 days	14 days	28 days
Control allografts	0/2	7/7	ND	ND
EFAD allografts	0/4	0/7	0/10	0/6
EFAD allografts plus				
EFAD spleen cells	ND	3/3	ND	ND
Control spleen cells	ND	2/2	ND	ND

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