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Pyelonephritis Transferred by Parabiosis[1] (38252)

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There seems little question that acute pyelonephritis is the direct consequence of bacterial invasion of the kidney. However, the nature of chronic pyelonephritis is a matter of dispute. Because of the frequent absence of bacteria from human kidneys where pathologic evidence indicated pyelonephritis (1), some investigators have raised questions as to whether persistent bacterial infection is a necessary prerequisite for chronic pyelonephritis (2, 3). On the other hand there is considerable evidence that bacteria do play a role in chronic pyelonephritis (4–6).

Earlier studies from this laboratory (7) suggested that in ascending pyelonephritis with *Streptococcus faecalis* progression occurred in the absence of bacteria. One possible explanation of progressive pyelonephritis with failure to isolate bacteria from pyelonephritic kidneys may be that bacteria initiate pyelonephritis, but once started, the process is self perpetuating, possibly immunologically mediated, and the presence of viable bacteria is no longer required. The present studies were designed to determine whether the histologic picture of pyelonephritis could be transferred to normal recipient animals by parabiosis with pyelonephritic animals.

Enterococcal hematogenous pyelonephritis was produced in inbred male Fischer rats as described previously (8). One ml of an overnight culture of *S. faecalis* in brain heart infusion broth (BHI) was injected intravenously. These rats were parabiosed[1] (9) to three groups of normal recipient rats. In group A the infection was eliminated by antibiotic treatment of pyelonephritic donors prior to parabiosis with the recipients. Infection was allowed to proceed for 12 weeks. Then benzathine penicillin (Bicillin, Wyeth) therapy was instituted, one injection of 600,000 units per week for 13 weeks followed by kanamycin, 5 mg twice a day for 3 weeks. The rats were parabiosed to their normal recipients 16 weeks from start of antibiotic therapy, 28 weeks from time of infection. In group B the animals were parabiosed at 28 weeks after infection without any prior antibiotic treatment. In group C the prior duration of infection was 8 weeks, and the animals did not receive antibiotic therapy. In all experiments control donor rats were injected with BHI and subsequently treated exactly the same as those donors which had received enterococci.

At a given time after parabiosis a unilateral nephrectomy for histology and bacterial assay was done on the recipient. The kidney was ground in 9 ml BHI and 1 ml plated in molten BHI agar as described previously (8). Later at sacrifice the remaining recipient kidney and both donor kidneys were examined. Thus recipient kidneys were examined at 1, 2, 3, 4, 8, and 12 weeks and donors at 3, 4 and 12 weeks after parabiosis. In all groups kidneys that did not conform to the expected bacteriologic status were excluded. These were kid-

[1] Fischer rats were obtained from Charles River Breeding Laboratories, Inc., Wilmington, MA, who confirmed histocompatibility of this strain of rats by skin grafting.

neys from untreated infected donor rats with no bacteria, antibiotic-treated donor rats which had residual bacteria, and control uninfected donors which had any bacteria. Any recipient with bacteria in the kidney was eliminated. The aberrant animals represented 7% of the total number examined.

A scoring system (0–4 plus) was used to grade the intensity of the pyelonephritis according to four major categories. Cortical mononuclear infiltrates started with 1 plus for widely scattered focal lesions to 4 plus for extensive confluent lesions covering 25% of the section. Interstitial tubular lesions of 1 plus consisted of focal increase of peritubular connective tissue increasing to 4 plus with more than two large superficial or deep scars involving more than 25% of the section. One plus calyceal lesions consisted of mild focal subcalyceal mononuclear infiltrate without epithelial hyperplasia, increasing to 4-plus score with extensive nodular hyperplasia and nodular lymphoid infiltration. Papillary 1-plus lesions were mild focal collecting duct hyperplasia with focal increase in connective tissue, increasing to 4 plus with extensive fibrosis, distortion, tubular atrophy and dilatation. The histologic examination of one section from each kidney was done blind by code number. Repeat blind reading of randomly selected slides established the reliability of the histologic examination. 100 randomly selected slides were examined a second time. Correlation between the two readings was $r = 0.87$, $p < .001$.

Lesions of chronic pyelonephritis described previously (10) were observed in the untreated and antibiotic treated *S. faecalis* infected donors.

The abnormalities in the recipients parabiosed to infected animals with or without treatment resembled those found in the donor infected animals. The cortical lesions consisted of superficial scars at times extending into deeper zones associated with tubular atrophy, peritubular basement membrane thickening and a variable degree of mononuclear cell infiltration (Fig. 1A). In areas of severe scarring, periglomerular fibrosis was also seen. Milder lesions con-

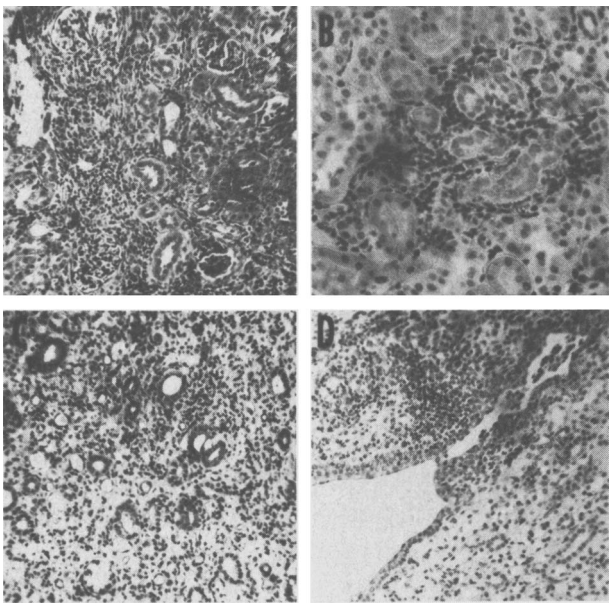

FIG. 1. (A) Cortex from a recipient parabiosed to an infected, treated donor for 12 weeks. (Hematoxylin-Eosin ×225) (B) Cortex from a recipient parabiosed to an infected, treated donor for 8 weeks. (H & E ×375) (C) Papilla from a recipient parabiosed to an infected, treated donor for 12 weeks. (H & E ×225) (D) Calyceal area at fornix from a recipient parabiosed to an infected, untreated donor for 4 weeks. (H & E ×225)

sisted of focal aggregation of mononuclear cells associated with tubular basement membrane thickening and tubular cytoplasmic alterations (Fig. 1B). Papillary lesions were focal and consisted of irregular increase in interstitial connective tissue, tubular atrophy and dilatation and scanty mononuclear cell infiltration (Fig. 1C). Calyceal lesions were noted principally in the recipients of infected and untreated donors (Fig. 1D). Widely scattered lesions principally confined to the cortex and consisting of focal accumulation of mononuclear cells around vessels and tubules were observed in both the normal, broth injected donors as well as the recipients parabiosed to these donors. Occasionally, prominent tubular cytoplasmic basophilia and vacuolization was observed in the antibiotic treated donors. Significant glomerular or vascular lesions were not noted in the control, broth injected donors or recipients.

Quantitation of the intensity of pyelonephritis in antibiotic treated donors and their recipients from group A is shown in Fig. 2A. The 4 parameters of pyelonephritis studied, mononuclear infiltration, interstitial calyceal and papillary lesions, are given equal weight and averaged. The maximum possible intensity (ordinate) is 4.0. The calyceal lesions contributed little to the overall evaluation; these disappeared after treatment in the donors and were not transferred to recipients. The intensity of pyelonephritis in donors declined over the period of observation from 3 to 12 weeks of parabiosis. In recipients of treated infected donors the intensity began at a low level, rose to about half of that found in the donors by 3 weeks and remained constant up to 12 weeks. In control animals, both donors and recipients, some lesions of minimal intensity were present. These findings in the control animals represent "background" natural lesions in the strain of animals used or lesions developing as a consequence of parabiosis.

The outcome of experiments in which animals had been parabiosed after 28 weeks of untreated infection (group B) is shown in Fig. 2B. These data are different from those previously shown with treated donors in two respects: there was an early marked

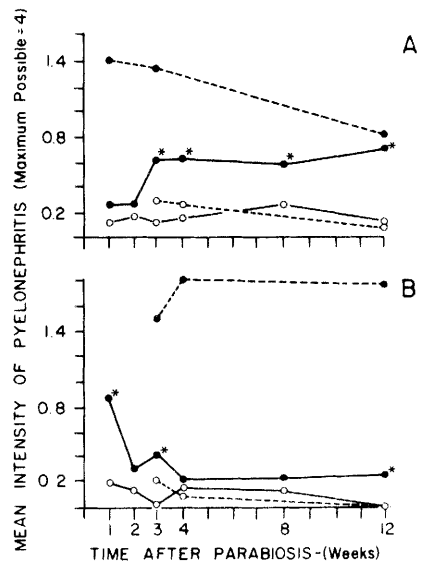

FIG. 2. Intensity of histologic changes in: (A) antibiotic treated donor pyelonephritic rats and their parabiont recipients parabiosed 28 weeks after infection, (B) untreated donor pyelonephritic rats and their parabiont recipients parabiosed 28 weeks after infection. ● Infected, ○ broth control, — recipient, --- donors, $*p < 0.05$ recipients of infected donors vs recipients of broth control donors.

response in recipients of untreated infected animals not present in recipients from treated infected rats. Secondly, intensity of lesions in recipients of untreated infected donors declined and leveled off at a significantly ($p < 0.05$) lower mean than in recipients of treated animals. Results obtained with parabiosis of animals with untreated 8 week pyelonephritis (group C), were similar to those obtained with the untreated 28 week infection.

The frequency of combined lesions was also analyzed (Table I). Animals were included if they had lesions $\geqq 1.0$ in any of the four parameters of pyelonephritis. In all groups, recipients of donors with pyelonephritis had significantly more lesions than control recipients.

The premise for this study was that there is an immunologic component to lesions observed in chronic pyelonephritis which is not dependent upon the continued presence of viable bacteria. The results indicate that some circulating factor, possibly immuno-

TABLE I. Frequency of Renal Lesions in Recipients of Parabiosed Animals.

Group	Donor infected	Donor treatment	Time of infection to parabiosis (weeks)	Proportion of recipients with lesions	
A	Yes	Yes	28	42/98	
	No (control)	Yes	28	20/105	$p < 0.005$
B	Yes	No	28	30/64	
	No (control)	No	28	12/85	$p < 0.005$
C	Yes	No	8	44/84	
	No (control)	No	8	36/112	$p < 0.05$

logic in nature, is present in animals with chronic pyelonephritis, which is capable of inducing a similar histologic lesion in a normal, isologous rat. Parabiosis has been successfully employed in passive transfer experiments in other models of immune tissue injury (11). It seems unlikely in view of the bacteriologic data that active induction of disease by transferred viable bacteria was responsible for the observed lesions. Although the parabionts were highly inbred, it is possible that minor degrees of histoincompatibility and resultant allograft immunity were responsible for the renal lesions. The differences between controls and infected donor recipient pairs could not be readily explained on this basis, unless infection exerts a nonspecific adjuvant effect intensifying an underlying allograft immunity or that antigens crossreactive with the putative minor transplantation antigens exist on the surface of *S. faecalis*. The fact that treatment intensified the lesions in recipients is unexplained. Although these results will require confirmation and extension in other systems and may be applicable only to the model described, they are consistent with the view that a cell mediated or humoral mediated immunity against normal kidney arises as a feature of bacterial tissue injury, or *S. faecalis* antigens crossreact with normal tissue antigens specific for kidney. Humoral antibody to kidney antigens could not be demonstrated in rats with hematogenous *S. faecalis* pyelonephritis (12), or in patients with chronic bacteriuria (13). We therefore favor the possibility that cell mediated immunity is responsible for the renal lesions in parabiosed rat.

Summary. Histologic changes resembling pyelonephritis developed in normal isologous rats parabiosed to rats with chronic enterococcal pyelonephritis. "Transfer" occurred even when viable bacteria had been eliminated from donor animals by antibiotic therapy. The "transferred" lesions, mononuclear cell infiltrate, tubular and papillary lesions, appeared at 3 weeks and persisted for 12 weeks (duration of study).

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