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BRIEF COMMUNICATIONS

Macrophage Chemotaxis in Anti-tubular Basement Membrane-Induced Interstitial Nephritis in Guinea Pigs¹

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Interstitial renal lesions containing T cells and macrophages develop after 14 days in guinea pigs immunized to produce anti-tubular basement membrane-induced interstitial nephritis. We serially examined the renal venous and systemic arterial sera from such animals to determine if chemotactic factors were released across their kidneys. Our findings demonstrated the presence of a macrophage-specific renal chemoattractant with peak detectability on Days 10-14, just subsequent to the deposition of α TBM-Ab, but prior to the development of significant renal injury. We propose that such factors may provide important communication signals in the immunopathogenesis of this form of interstitial injury. © 1985 Academic Press, Inc.

INTRODUCTION

Guinea pigs injected with renal tubular basement membrane antigen (RTA)⁶ in adjuvant develop anti-tubular basement membrane antibodies (α TBM-Ab) by 7-10 days, and severe interstitial nephritis after 14 days (1). The developing or fully formed interstitial lesion does not involve neutrophils (2, 3), but rather contains a variety of mononuclear cells (2, 3), including macrophages (2, 4) and T cells (3) in association with kidney-bound α TBM-Ab and complement (5, 6). The interstitial lesions in guinea pigs can be transferred with α TBM-Ab (7), but not with cells (2). Such α TBM-Ab can also form an informational bridge between the tubular basement membrane and natural killer cells (8). Progressive interstitial

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⁶ Abbreviations: RTA, renal tubular antigen; CFA, complete Freund's adjuvant; α TBM-Ab, anti-tubular basement membranes antibodies; CI, chemotactic index; ZAS, zymosan-activated serum; FITC, fluorescein isothiocyanate.

disease is furthermore associated with T-cell-dependent fibrogenesis modulated by lymphokines (9, 10).

While α TBM-Ab seems to be sufficient to initiate interstitial injury in guinea pigs (2, 7), we wished to further examine the premise that a renal gradient of chemoattraction might also be generated for the trafficking of macrophages into the interstitium of nephritic kidneys.

MATERIALS AND METHODS

Animals and immunization. Hartley guinea pigs were immunized with 2 mg of RTA in complete Freund's adjuvant (CFA). Control guinea pigs were immunized with CFA alone. The RTA was isolated by differential sieving, and sonicated, lyophilized, and stored at -70°C (1).

Preparation of serum for chemotaxis. Serum samples were obtained from the renal vein and descending lower aorta of anesthetized guinea pigs 7, 10, 14, and 17 days after immunization. The blood was gently withdrawn and allowed to clot for 60–90 min; the serum was stored at -70°C . The kidneys were removed afterward for histologic examination.

Measurement of chemotaxis. The chemotaxis assay was performed using a standard procedure as previously described in blind-well chambers (11). Guinea pig peritoneal mononuclear cells (85–90% macrophages) were obtained 96 hr after stimulation with mineral oil. Neutrophils were elicited after the intraperitoneal injection of glycogen. Optimal running conditions of the chemotaxis assay included a 90-min incubation at 37°C in room air using a cell concentration of $2.5\text{--}3.5 \times 10^6$ cells/ml of Gey's buffered salt solution with 2% bovine serum albumin (pH 7.2). Zymosan (10 mg/ml)-activated normal guinea pig serum (ZAS) was used as a positive chemoattractant control. Experiments were run in duplicate or triplicate using three to five animals/data point. Chambers with no concentration gradient for chemoattractant were routinely run to check for nondirectional chemokinesis (12). Chemotactic migration was measured by counting the total number of cells (10 oil immersion fields) completely transversing a $5\text{-}\mu\text{m}$ polycarbonate filter (Millipore, Bedford, Mass.) into wells containing test serum for chemoattraction. The possibility of cells crossing the filter and dropping into the lower chamber was excluded in each experiment. The chemotactic activity was then expressed as a mean chemotactic index (CI) = (chemotaxis – chemokinesis of the test serum)/(chemotaxis – chemokinesis of 10% ZAS).

Assessment of renal disease. Kidney tissue was prepared for direct immunofluorescence and light microscopy by standard methods for this laboratory (1). The presence of α TBM-Ab and the degree of cortical interstitial involvement was determined from coded sections using a grading scale (0 to 4) previously described in detail (1, 13).

RESULTS

The existence of *in vivo* chemotactic activity might be inferred from the observation of late migration and accumulation of macrophages into the renal interstitium of nephritic guinea pigs 14–21 days after immunization with RTA/CFA

TABLE 1
DEVELOPMENT OF α TBM-Ab AND INTERSTITIAL LESIONS IN GUINEA PIGS

Day ^a	Kidney-bound α TBM-Ab ^b		Histology ^c	
	RTA/CFA	CFA	RTA/CFA	CFA
7	1.5 \pm 0.5	0.0	0.0	0.0
14	3.8 \pm 0.2	0.0	0.5 \pm 0.3	0.0
17	4.0 \pm 0.0	0.0	3.3 \pm 0.5	0.0

^a Days after immunization.

^b Direct immunofluorescence with FITC anti-guinea pig IgG.

^c Histologic grading of cortical interstitial lesions ($n = 3-5$ /group).

(1-3). A similar pattern of injury was observed in the current cohorts of animals; that is, α TBM-Ab was present by 7 days and significant mononuclear cell lesions routinely appeared after 14 days (see Table 1).

The chemotactic activity of renal venous blood was initially examined 14 days after immunization (see Fig. 1). We observed a direct relationship between the

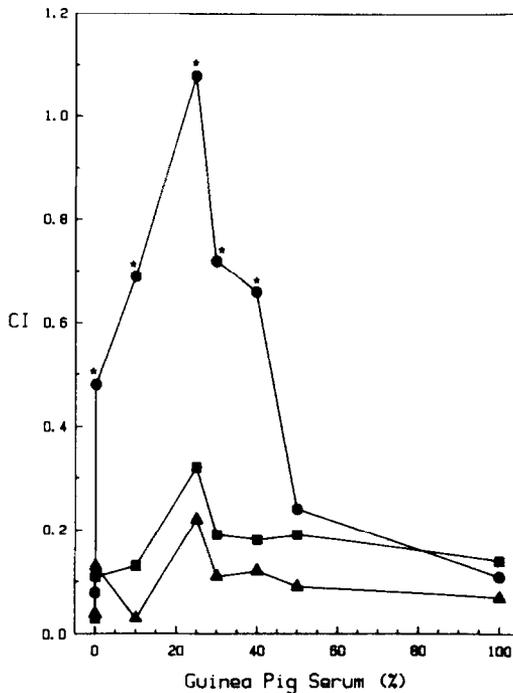


FIG. 1. Effects of serial renal venous serum dilutions on the chemotaxis of peritoneal macrophages. Serum was obtained 14 days ($n = 3$ /group) following immunization with RTA/CFA (●), CFA (■), or saline (▲), and chemotaxis was expressed as a mean chemotactic index (CI). 10% ZAS produced chemotaxis = 18.1 ± 1.0 and chemokinesis = 1.1 ± 0.3 . SEM were less than 7% of the means. The results indicate that chemotactic activity can be detected in the renal venous effluent at low serum concentrations (0.1-40%) in guinea pigs immunized with RTA/CFA. This activity disappears at higher concentrations of serum and was not present at all in controls. (*) $P < 0.001$ compared to normal serum control.

chemotactic index and the percentage of renal venous serum added from nephritic guinea pigs. This dose-response relationship existed over a range of lower serum concentrations (0.1–40%; $P < 0.001$), but fell off at higher concentrations (14). Chemotactic activity was not significantly present in controls immunized with CFA nor in saline-injected animals providing normal guinea pig serum.

A temporal comparison of chemotactic activity from systemic arterial and renal venous serum is presented in Fig. 2. Renal venous serum samples (25% dilution) showed peak chemotactic activity between 10 and 14 days ($P < 0.001$), just before the accumulation of mononuclear cells in the kidney. On Day 10 the arterial serum also demonstrated increased chemotactic activity ($P < 0.008$), but less than that observed from the renal venous system ($P < 0.001$), suggesting that cell chemoattractants were released across the kidney. This chemoattractant effect was short lived, however, and largely dissipated in arterial serum by Day 14, and in renal venous serum by Day 17.

The specificity of this renal chemoattractant was analyzed by comparing its effect on both peritoneal macrophages and neutrophils (see Table 2). Pooled Day

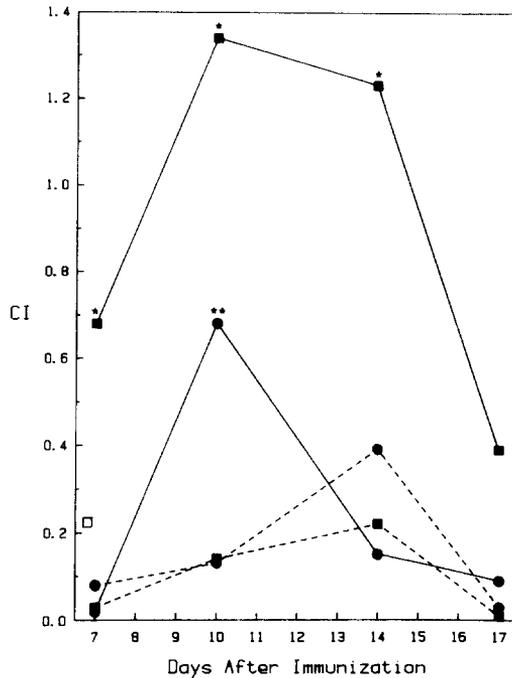


FIG. 2. Serial day comparisons of renal venous and systemic arterial sera on the chemotaxis of peritoneal macrophages. Renal venous (■) and systemic arterial (●) sera were obtained on Days 7, 10, 14, and 17 ($n = 3-5/\text{group}$) following immunization with RTA/CFA (—) or CFA (---). Normal guinea pig serum (□). Results were expressed as a mean CI. 10% ZAS produced chemotaxis = 17.8 ± 0.7 and chemokinesis = 1.0 ± 0.2 . SEM were less than 7% of the means. The results indicate that chemotactic activity was strongly present in the serial venous serum from RTA/CFA animals on Days 10–14. Chemotaxis with systemic arterial serum from RTA animals could only be demonstrated on Day 10; this activity, however, was significantly less than that detected in the renal venous effluent. (*) $P < 0.001$ and (**) $P < 0.008$ when compared to normal serum.

TABLE 2
CHEMOATTRACTANT SPECIFICITY OF NEPHRITIC SERUM

Groups	Chemoattractants				
	10% ZAS	Nephritic serum ^a			
		25%	10%	1.0%	0.1%
A. Macrophages					
Chemotaxis	18.6 ± 0.8	22.0 ± 1.0	14.8 ± 0.7	9.8 ± 0.8	3.4 ± 0.4
Chemokinesis	1.1 ± 0.2	0.0 ± 0.0	1.1 ± 0.3	0.6 ± 0.3	0.0 ± 0.0
Chemotactic Index (CI)		1.3	0.8	0.5	0.2
B. Neutrophils					
Chemotaxis	14.5 ± 0.4	0.8 ± 0.3	0.5 ± 0.2	0.05 ± 0.05	0.1 ± 0.07
Chemokinesis	0.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1
Chemotactic Index (CI)		0.06	0.03	0.0	0.0

^a Pooled renal venous serum from guinea pigs bled 14 days after immunization with RTA/CFA.

14 nephritic renal venous serum was strongly chemotactic for macrophages at a 10–25% serum dilution, but had no effect on neutrophil migration. These neutrophils, however, were responsive to 10% ZAS.

DISCUSSION

The precise mechanisms by which T cells and macrophages migrate into the interstitial lesions of anti-tubular basement membrane disease are only partially defined in the guinea pig. The passive transfer of α TBM-Ab into bone marrow-reconstituted recipients demonstrates a minimum requirement of an intact bone marrow for the expression of this disease (4). Sensitized T cells can directly migrate to normal kidneys within 24 hr of adoptive transfer (15) and, while macrophages appear after the passive transfer of α TBM-Ab into C4-deficient guinea pigs (5), lesions do not develop in recipients pretreated with cobra venom factor (6), indirectly suggesting that macrophage recruitment may depend on the alternate complement pathway. The presence of complement, however, has not always been detectable in these renal lesions (3), and its definitive role is still uncertain.

Our findings in this model suggest that a chemoattractant is released across the kidney shortly after the appearance of α TBM-Ab, but just prior to the full development of interstitial lesions. This chemoattractant was specific for macrophages. Because commonly described factors, such as C5a (16), some lymphokines (17), protease-generated factors from IgG (18), and N-formulated oligopeptides (19), are chemotactic for both macrophages and neutrophils, our finding of a macrophage-specific effect is somewhat unique (20), but consistent with the absence of accumulating neutrophils in the interstitial lesions of nephritic guinea pigs (2, 3).

The molecular nature of this chemoattractant effect remains unknown and needs to be further investigated. Preliminary efforts to characterize this factor (data not shown) suggest that it is heat stable at 56°C, and is probably not a heat-labile complement component. Nor does it seem to be a T-cell lymphokine, as tested from the culture supernatants of tubular antigen-activated lymphocytes. The kinetics of the effect and the gradient across the kidney lend support to a hypothesis, however, that early immunologic events (perhaps kidney-bound

α TBM-Ab, with or without the alternate complement pathway (6)), may initiate the release of active moieties which subsequently attract macrophages into developing interstitial lesions.

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