Local Production of Antibodies to Leptospira pomona in Kidneys of Chronically Infected Skunks (Mephitis mephitis)*

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Local Production of Antibodies to *Leptospira pomona* in Kidneys of Chronically Infected Skunks *(Mephitis mephitis)**

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Abstract

The kidneys of skunks chronically infected with *Leptospira pomona* contained a conspicuous number of plasma cells. From comparative studies of overall gammaglobulin content and specific antibody titers in sera and kidney extracts it was concluded that specific antibodies to *L. pomona* were synthesized in the kidneys of skunks with renal leptospirosis.

Introduction

The presence of specific antibody in the urine is a common finding in leptospiral infections of man[9,11] and other mammals.[2,3,5,6,7,10,11] The presence of urinary antibodies in mice with *Leptospira australis B* infection was found to be related to renal damage.[5] Thus, the presence of antibodies in the urine appeared to result from overflow from the plasma.[5] However, in pigs experimentally infected with *Leptospira pomona* urinary antibodies were not found when kidney damage was greatest, ie. two to four weeks post-infection, but they were detected at about three months after infection.[7] Similar observations were made in experimental infections in skunks[10] indicating that the urinary antibody might have been synthesized locally in the urinary tract.

The present study was undertaken to investigate if antibodies to *L. pomona* were produced locally in the kidneys of chronically infected skunks. This was done by a comparative study of antibody titers to *L. pomona* and overall gammaglobulin contents in sera and kidney extracts of the test animals. In addition, a histological examination of the kidneys was carried out.

Materials and Methods

Four groups of skunks were studied: (1) three control animals which had no serum titers to *L. pomona*, (2) four control animals which had passively received antibodies to *L. pomona*, (3) ten skunks which were experimentally infected with *L. pomona* and (4) five skunks which were found to be naturally infected with *L. pomona*.

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Source of animals: The skunks used as controls were raised at the mink ranch of the Ontario Veterinary College. All other skunks used were captured in the wild. Before the start of the experiments, a serum sample from each animal was examined for leptospiral antibodies. *Leptospira pomona, L. icterohaemorrhagia, L. canicola, L. grippotyphosa,* and *L. sejroe* were employed as antigens. Most of the skunks, except the ones found naturally infected, had been surgically descented.

Control animals: Three normal skunks were used as negative controls. Another four skunks which were serologically negative for *L. pomona* were intravenously injected with 10 ml of bovine serum containing a high antibody titer to *L. pomona.* They were exsanguinated 24 hours after the administration of the specific antiserum.

Infection: Ten skunks were experimentally infected with a strain of *L. pomona* originally isolated from a cottontail rabbit *(Sylvilagus floridanus)*[10]. They were inoculated intraperitoneally with approximately 10^4 organisms and exsanguinated at various times post-inoculation (p i).

Five skunks used for the experiments had been naturally infected with *L. pomona,* as demonstrated by specific serum antibodies, and by the isolation of the organisms from the kidney or the urine of three of them.

Collection and processing of specimens: The skunks, anesthetized by ether, were bled from the heart with a 50 ml syringe. As much blood as obtainable was withdrawn. The kidneys were removed aseptically and kidney tissues were cultured for leptospires.

Kidney extract was prepared as follows:

Pieces of kidney cortex of the infected and the control animals were minced with scissors. Small amounts of the kidney pulp were added to a graduate containing saline to yield a kidney suspension in a dilution of 1:5. The suspension was ground in a Ten Broeck tissue grinder and then centrifuged at 800 g. The supernatant fluid represented the kidney extract tested for antibody activity to *Leptospira pomona.*

Urine collected from the bladder and the kidney suspension of each skunk were cultured for leptospires to determine if the animals were renal carriers at the time of exsanguination.

Cultural methods: Urine and kidney suspensions were cultured in Korthof's medium (1) as follows: 0.3 ml of a specimen was added to 3 ml of medium and then serially diluted three times to give a dilution of about 10^4 in the final tube.

Serology: Sera and kidney extracts of the skunks were stored at —20C until tested. The microscopic agglutination test with live antigen was used to demonstrate antibody levels (4). Antigens were maintained in Korthof's medium. The serum and kidney extract of each animal were tested simultaneously using the same batch of antigen. The serum was tested in the following dilutions: 1:2, 1:5, 1:10, 1:25, 1:50 and continuing in two-fold dilutions. The kidney extract was tested in two-fold dilutions, starting with a dilution of 1:10.

Histology: A piece of kidney of each animal was fixed in a 10 percent solution of buffered formalin. Sections of the kidneys were stained with hematoxylin and eosin or with hematoxylin, phloxin and safranin.

Immunoelectrophoretic analysis of sera and kidney extracts: An antiserum to skunk whole serum was produced in a rabbit by repeated subcutaneous injections of an emulsion of skunk serum and complete Freund's adjuvant.

Immunoelectrophoresis was carried out according to standard procedures.[8] Immunoelectrophoretic patterns of sera and kidney extracts were obtained by the use of the antiserum to skunk whole serum. This procedure allowed a semiquantitative evaluation of the relative gammaglobulin concentrations in the sera and kidney extracts.

Results

Histopathology of kidneys of infected skunks: Skunks 1 and 2 (22 days p i) had cloudy swelling and vacuolar degeneration of the convoluted tubules. Some degree of tubular necrosis was present.

In experimentally infected skunks kept for a longer time p i (80 days or more) the pathological changes were characterized by infiltration with lymphoid cells and histocytes of variable degree. Some foci of infiltration contained a conspicuous number of plasma cells (Fig. 1). These infiltrations were either scattered over the cortex in small foci, or in streaks reaching from the capsule to the zone of arcuate vessels. Varying degrees of necrosis of tubules was found.

The naturally infected skunks had similar renal lesions. Interstitial nephritis was pronounced in skunks 13 and 14. Skunk 14 had dilated tubules with hyalin casts. Some tubules had undergone complete necrosis and were replaced by connective tissue.

Immunoelectrophoresis: The serum and kidney extract of each skunk were compared by immunoelectrophoresis. In all skunks, considerably stronger precipitation arcs of γ-globulin were observed in the sera than in the kidney extracts (Fig. 2).

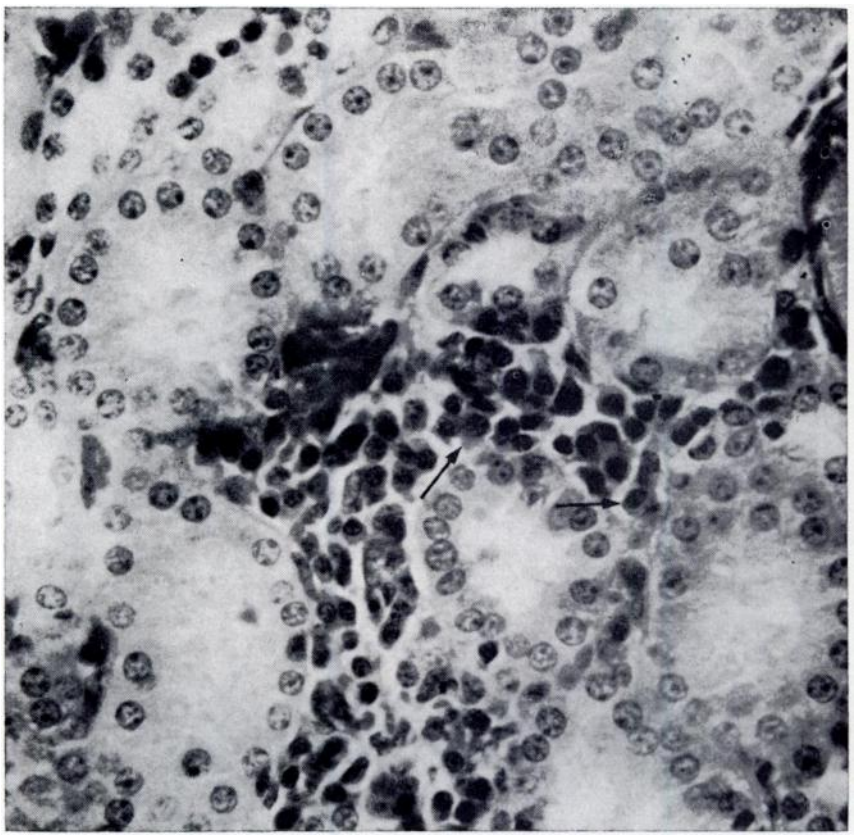

FIGURE 1. *Lymphoid cells in the renal cortex of skunk #15. Note the presence of large lymphocytes and plasma cells (arrows).*

Specific antibody in sera and kidney extracts: The three control animals which had no detectable antibodies to *Leptospira pomona* in their sera also had no detectable antibodies in their kidney extracts (Table 1).

The four control animals which had passively received antibodies to *L. pomona* had considerably higher antibody titers in the serum than in the kidney extract. The ratio of kidney titer to serum titer was 1:16 in two animals and 1:40 in the other two animals (Table 1). Thus, the average ratio of kidney titer to serum titer in the animals with passively acquired antibodies was 1:28.

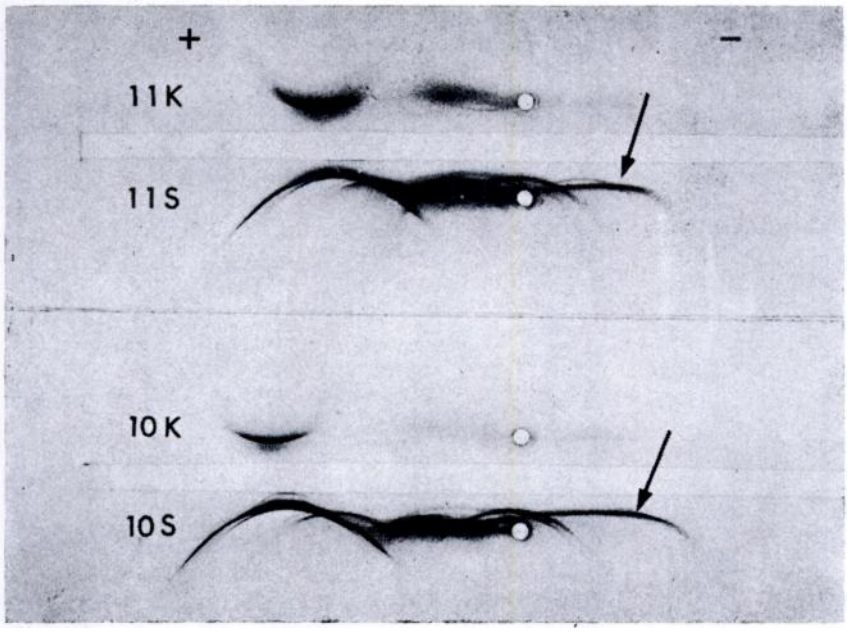

FIGURE 2. *Immunoelectrophoresis of serum (S) and kidney extract (K), both in dilution of 1:5 of skunk #11 and #10. The troughs contained rabbit antiserum to skunk whole serum. Note the stronger precipitin arc of the serum gammaglobulin (arrows).*

TABLE 1. *Antibody titers to* Leptospira pomona *in kidney extracts and sera of normal skunks injected intravenously with specific antisera to* L. pomona.

Skunk No.	Antibody titer to *L. pomona* Kidney extract	Serum	Ratio of kidney titer to serum titer
1 C *	< 10	< 2	
2 C *	< 10	< 2	
3 C *	< 10	< 2	
4 C	200	3200	1:16
5 C	25	400	1:16
6 C	10	400	1:40
7 C	10	400	1:40

* Controls, not injected.

TABLE 2. *Antibody titers to* Leptospira pomona *in kidney extracts and sera of infected skunks.*

Skunk No.	Antibody titer to *L. pomona* Kidney extract	Serum	Ratio of kidney titer to serum titer	Days post-inoculation
1 *	25	1600	1:64	22
2 *	100	6400	1:64	22
3 *	< 10	50	< 1:5	197
4 *	400	1600	1:4	N.I. **
5 *	6400	12800	1:2	197
6	400	800	1:2	197
7 *	800	1600	1:2	N.I.
8 *	800	1600	1:2	N.I.
9 *	1600	1600	1:1	190
10 *	12800	12800	1:1	104
11 *	10	10	1:1	80
12	< 10	5	< 2:1	104
13	1600	800	2:1	N.I.
14	400	200	2:1	N.I.
15 *	6400	3200	2:1	190

* *L. pomona* cultured from kidney or urine of the exsanguinated animal.
** Natural infection.

The 15 infected skunks which had actively synthesized antibody to *L. pomona*, had varying ratios of kidney to serum antibody titers (Table 2).

Two skunks (1 and 2) had a 1:64 ratio of kidney to serum antibody titer (Table 2), ie. a smaller ratio than that found in the control skunks which had passively received specific antibodies.

A higher ratio of kidney to serum antibody titer was found in all other skunks when compared to the four control animals which had passively received antibodies (Table 2). One animal had a ratio of kidney to serum antibody titer of 1:4. In four animals this ratio was 1:2. In three animals the kidney antibody titers were as high as the serum antibody titers and in three other animals the kidney titers were even greater than the serum titers.

Carrier state: Eleven of the 15 infected skunks were found to be leptospiral carriers at the time of exsanguination, as demonstrated by isolation of leptospires from the urine or the kidneys (Table 2). Skunks 1 and 2 were shedding considerable numbers of leptospires in the urine. Leptospires could be detected in these two skunks by examination of the urine under the darkfield microscope.

Discussion

The immunoelectrophoretic analysis revealed that the sera of all skunks contained higher concentrations of gammaglobulin than the kidney extracts.

In the skunks which had passively received antibodies to *L. pomona* the average ratio of kidney antibody titers to serum antibody titers was 1:28. This is taken as a quantitative indicator for the relative amount of overall gammaglobulin in the kidney due to the remnants of serum globulins in the blood vessels.

With the exception of skunks 1 and 2, all skunks which had actively produced antibody to *L. pomona* had higher ratios of kidney antibody titers to serum antibody titers than the control animals which had received antibodies passively. Some of the skunks had as high a specific antibody activity in the kidney extract as in the serum, and in three animals the antibody activity was even higher in the kidney extract than in the serum.

These high kidney antibody titers could not be explained by serum overflow due to renal damage, since the overall gammaglobulin content was lower in the kidney extract than in the serum, but the specific antibody titer in some infected animals was as high or higher in the kidney extract than in the serum. Therefore, it is concluded that specific antibodies were locally synthesized in the kidneys. This conclusion is supported by the finding of plasma cells in the kidney cortex. It is also in concordance with the finding that the appearance of dectectable amounts of antibodies to *L. pomona* in the urine did not coincide with the period of proteinuria (8 to 50 days p i).[10] Urinary antibodies in titers of 1:100 were demonstrable 170 days p i while there was no detectable proteinuria.[10]

One has to keep in mind that leptospires were isolated from most of the skunks. In these cases, the antibody activity of the kidney extracts was probably reduced by the specific absorption of antibody by the renal leptospires. This fact may well account for the relatively low antibody activity demonstrated in the kidney extracts of skunks 1 and 2 which shed large numbers of leptospires in the urine.

From the presented data it is obvious that locally produced antibody was not able to completely eliminate the renal leptospires in most of the skunks. It is, however, conceivable that specific antibodies have a limiting effect on the spreading of localized infection, ie. reinvasion of leptospires from the lumen of the tubules into the blood stream.

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