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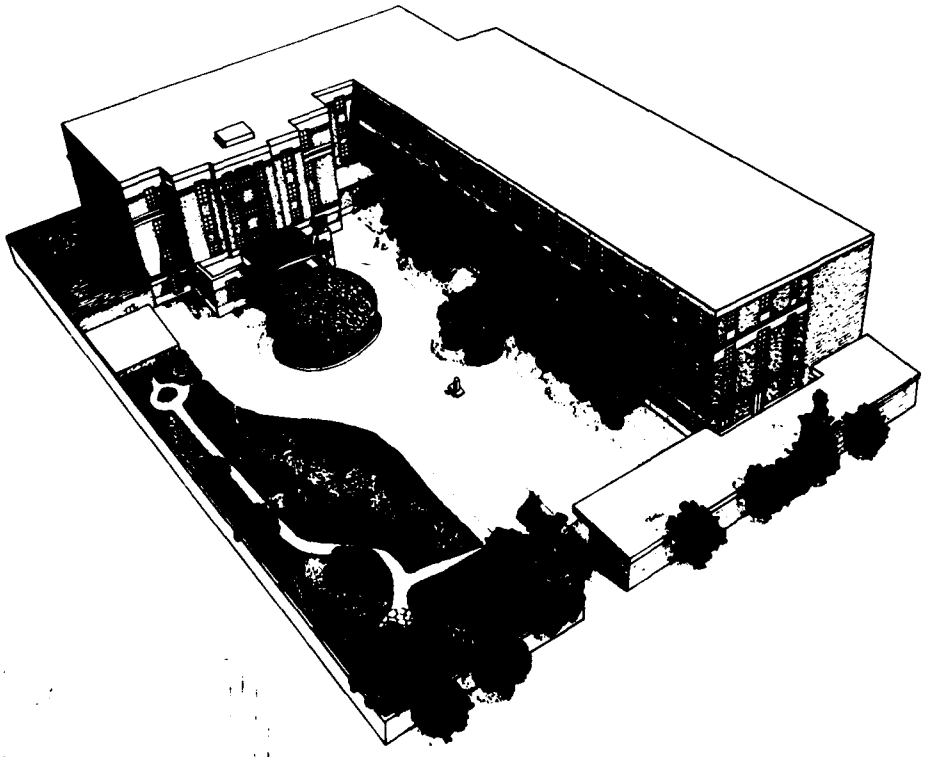
REPORT OF THREE CASES



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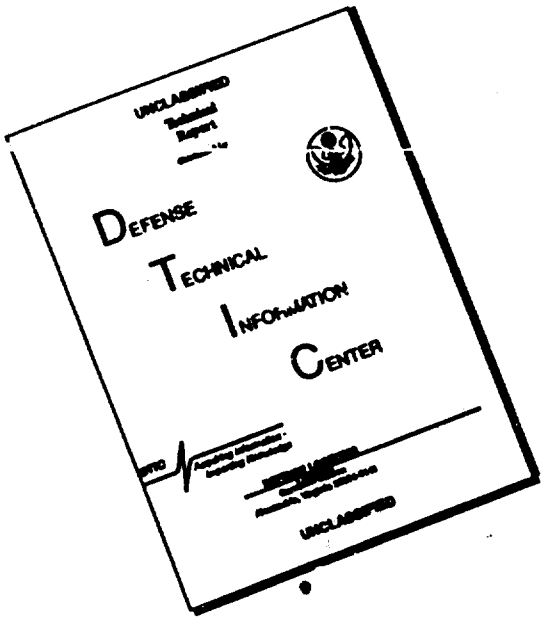

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J-J. GUNNING, A.B., M.D., HEAD

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R. H. WATTEN
CAPT MC USN
COMMANDING OFFICER

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MYRON J. TONG, EUGENE B. ROSENBERG, BERNHARD A. VOTTERI, AND CHE-CHUNG TSAI

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13 ABSTRACT

IN THREE PATIENTS WITH LEPTOSPIROSIS, IMPROVEMENT OF CLINICAL SYMPTOMS WAS CLOSELY ASSOCIATED WITH THE APPEARANCE OF AGGLUTINATING, COMPLEMENT-FIXING, AND HEMOLYTIC ANTIBODIES IN THE CIRCULATION. ELEVATIONS IN THE SERUM CONCENTRATIONS OF IgG, IgA, AND IgM WERE ALSO DEMONSTRATED. IN ONE PATIENT, RECURRENCE OF FEVER AND MENINGITIS DURING THE IMMUNE PHASE COINCIDED WITH THE APPEARANCE OF AGGLUTININS AND INCREASES IN THE LEVELS OF ALL THREE IMMUNOGLOBULINS IN THE SPINAL FLUID. ULTRACENTRIFUGATION OF IMMUNE SERA IN SUCROSEDENSITY GRADIENTS SHOWED THAT IgM WAS THE PREDOMINANT IMMUNOGLOBULIN INVOLVED IN THE AGGLUTINATION, COMPLEMENT-FIXATION, AND HEMOLYTIC TESTS. ALTHOUGH ANTIBODIES ARE IMPORTANT IN THE DEVELOPMENT OF IMMUNITY DURING THE SEPTICEMIC PHASE, ITS ROLE IN THE PATHOGENESIS OF CLINICAL SYMPTOMS DURING THE IMMUNE PHASE IS STILL UNCLEAR.

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IMMUNOLOGICAL RESPONSE IN LEPTOSPIROSIS*

REPORT OF THREE CASES

MYRON J. TONG,† EUGENE B. ROSENBERG,‡
BERNHARD A. VOTTERI,§ AND CHE-CHUNG TSAI¶
U.S. Naval Medical Research Unit No. 2 Detachment and the Department of Medicine, Naval Support Activity Hospital, DaNang, Republic of Vietnam

ABSTRACT: In three patients with leptospirosis, improvement of clinical symptoms was closely associated with the appearance of agglutinating, complement-fixing, and hemolytic antibodies in the circulation. Elevations in the serum concentrations of IgG, IgA, and IgM were also demonstrated. In one patient, recurrence of fever and meningitis during the immune phase coincided with the appearance of agglutinins and increases in the levels of all three immunoglobulins in the spinal fluid. Ultracentrifugation of immune sera in sucrose-density gradients showed that IgM was the predominant immunoglobulin involved in the agglutination, complement-fixation, and hemolytic tests. Although antibodies are important in the development of immunity during the septicemic phase, its role in the pathogenesis of clinical symptoms during the immune phase is still unclear.

(Accepted 1 February 1971)

Leptospirosis, a disease endemic in most parts of Southeast Asia, has recently been recognized as an important cause of febrile illnesses in American military personnel assigned to these areas.[1] The availability of improved laboratory facilities for isolation and serological identification of the infecting agents as well as early clinical recognition have allowed further studies into the pathogenesis of many important aspects of this disease.

Leptospirosis is often characterized by a biphasic clinical course. The manifestations of the primary or "septicemic" phase is attributed to leptospiremia, and improvement of clinical symptoms is closely associated with the appearance of serologically detectable antibodies.[2] After an asymptomatic period of 1 to 3 days, the onset of the secondary or "immune" phase may be heralded by the recurrence of fever, meningitis, and neurological abnormalities. These symptoms have been regarded as immunological responses of the host to infection and may be mediated by antigen-antibody reactions.[3-5] This study demonstrates the immunoglobulin response and the production of agglutinating, complement-fixing, and hemolytic antibodies during the septicemic and immune phases of leptospirosis.

MATERIALS AND METHODS

Patients admitted to the Naval Support Activity Hospital, DaNang, Republic of Vietnam, with symptoms of fever, headache, myalgia, conjunctival suffusion, and who were suspected of having leptospirosis were selected for study. These laboratory tests were performed on admission and on the 10th to 11th and 20th to 21st days of hospitalization: hematocrit, white blood-cell (WBC) count with differential, erythrocyte sedimentation rate (Wintrobe method), bilirubin, serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (Bodansky method), serum-protein electrophoresis, VDRL, blood urea nitrogen, creatinine, and urinalysis. Serum IgA (normal

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† Los Angeles County-USC Medical Center, Los Angeles, California 90033.

‡ Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014.

§ Department of Medicine, Wadsworth Veterans Administration Hospital, Los Angeles, California 90073.

¶ U.S. Naval Medical Research Unit No. 2, Taipei, Taiwan.

value 158 mg% ± 76), IgG (1,073 mg% ± 273), IgM (94 mg% ± 47), and the C'3 component of complement (166 mg% ± 60) were measured in Hyland Immunoplates by the radial immunodiffusion technique.[6] Immunoglobulins were also measured in the cerebrospinal fluid (CSF). The normal range for IgG in the CSF is 0.8% to 3.5 mg%; IgA and IgM are not usually detected.[7] Blood, urine, and CSF were inoculated into Fletcher's media for leptospiral culture.[8] The microagglutination, complement-fixation, and hemolytic tests for leptospirosis were performed according to the methods described.[9-11] Sera obtained from patient Number 1 on the 3rd, 12th, and 21st days of illness were subjected to ultracentrifugation at 35,000 rpm (85,000 × G) for 18 hours in a 10% to 40% sucrose-density gradient.[12] Each 0.25-ml sample of serum was diluted with 0.25 ml of normal saline solution and applied to the top of the gradient. After centrifugation, 10 fractions of 0.5 ml each were collected from the bottom of the tube with a 21-gauge needle. Each fraction was assayed for immunoglobulin concentration, microagglutination, complement-fixation, and hemolytic antibodies.

RESULTS

The case reports of three patients who were hospitalized with clinical symptoms of less than 3 days' duration and in whom the diagnosis of leptospirosis was later confirmed by either serology or isolation of the infectious agent are presented.

Case 1

A 19-year-old American marine was admitted to the Naval Support Activity Hospital in Da Nang, Republic of Vietnam, with fever, headache, nausea, and generalized myalgia. He had been on patrol in a swampy area 9 days before the onset of symptoms and felt well until 2 days before admission when he experienced the abrupt onset of severe occipital headache, photophobia, chills, and fever to 102°F. He also complained of intense pain on moving his neck.

On admission, the patient appeared acutely ill with a temperature of 100°F, blood pressure of 120 systolic, 80 diastolic, and pulse rate of 110 per minute. Pertinent physical findings included bilateral conjunctival injection with petechial hemorrhages in the sclerae, and nuchal rigidity. He had generalized muscle tenderness of the back and abdomen and complained of severe pain on palpation of the muscles of the lower extremities, especially the gastrocnemii. The remainder of the physical examination was normal.

Laboratory data on admission revealed a hematocrit of 43%, corrected erythrocyte sedimentation rate of 36 mm per hour, and a WBC count of 8,000 mm^3 with 83% polymorphonuclear neutrophils, 8% band forms, 6% lymphocytes, and 3% monocytes. Urinalysis showed a specific gravity of 1.020, *p*H 5.0, 20 mg% protein, and microscopic hematuria. The total bilirubin was 2.7 mg% with a direct fraction of 0.4 mg%, SGOT 92 units, prothrombin time 84%, and Lee White clotting time 18 minutes and 30 seconds. Total serum protein was 6.3 g, with albumin 3.75 g, $alpha_1$ globulin 0.39 g, $alpha_2$ globulin 0.65 g, beta globulin 0.58 g, and gamma globulin 0.93 g per 100 ml. The blood urea nitrogen was 19 mg and the serum creatinine 1.5 mg per 100 ml. Examination of the spinal fluid showed a glucose of 80 mg and protein of 43 mg per 100 ml; no cells were seen. Frequent malaria smears were negative for parasitemia, and the microagglutination, complement-fixation, and hemolytic tests for leptospirosis were negative when the patient was admitted. No organisms were seen on darkfield examination of the blood, urine, and CSF.

The patient continued to have daily oral temperature elevations to 103°F, headache, meningismus, and myalgia for 5 days. His symptoms then gradually subsided and he was essentially well 9 days after the onset of illness. However, on the 12th day, he again experienced a 36-hour episode of malaise and neck tenderness that was associated with a temperature of 99.6°F. Laboratory examination at this time showed a hematocrit of 41%, a WBC count of 8,065 mm^3 with 66% neutrophils, and an erythrocyte sedimentation rate of 34 mm per hour. The SGOT was 88 units, SGPT 87 units, blood urea nitrogen (BUN) 40 mg%, and the 24-hour creatinine clearance was 33 ml per minute. A repeat spinal fluid examination revealed 117 lymphocytes and 39 polymorphonuclear neutrophils, glucose 58 mg, and protein 37 mg per 100 ml. At this time, the serum and spinal fluid microagglutination titers for *Leptospira autumnalis* was positive at 1:3,200 and 1:200 respectively. The serum complement-fixation titer was 1:256, and the hemolytic titer was 1:2,560. Similar titers were obtained on the 21st

TABLE 1
Serological response in leptospirosis

Patient No.	Day of illness	Infecting organism	Microagglutination* titer		Complement-fixation† titer		Hemolytic test‡ titer	
			Serum	Spinal fluid	Serum	Spinal fluid	Serum	Spinal fluid
	3		0	0	0	1:8	1:40	0
1	12	*L. autumnalis*	1:3,200	1:200	1:256	1:8	1:2,560	0
	21		1:1,600		1:128		1:640	
	2		0	0	1:16	1:8	1:40	0
2	11	*L. pyrogenes*	1:400	0	1:512	1:16	1:640	0
	20		1:200		1:256		1:160	
	2		0	0	0	1:8	1:40	0
3	11	*L. bataviae*	1:1,600	1:200	1:128	1:8	1:640	0
	20		1:3,200		1:512		1:10,240	

* 1:100 titer significant.
† 1:16 titer significant.
‡ 1:160 titer significant.

day (Table 1). *L. autumnalis* was subsequently isolated on Fletcher's medium from blood and CSF obtained the 3rd day of illness and again from the CSF on the 12th day.

Immunoglobulin studies revealed an increase in the IgG, IgA, IgM, and the C'3 component of complement on the 12th day (Table 2). Sera obtained on the 3rd, 12th, and 21st days were subjected to ultracentrifugation in a 10% to 40% sucrose-density gradient. No agglutination, complement-fixation, or hemolytic activity was detected in the serum obtained the 3rd day. In the 12th-day sample, the highest microagglutination, complement-fixation, and hemolytic titers for *L. autumnalis* were found in fractions 3 and 4, which also contained the highest concentrations of IgM (Fig. 1). Serological titers were not detected in fraction 6, which contained the highest concentration of IgG and IgA. Similar results were found after ultracentrifugation of the sucrose-density gradient of sera obtained on the 21st day of illness.

Case 2

An 18-year-old man was admitted to the hospital with fever and chills of 1-day duration, severe myalgia, especially of the extremities and neck, photophobia, and headache. Nine days before onset of symptoms, he had been on patrol and walked through rice paddies near the demilitarized zone.

Physical examination on admission revealed a temperature of 103.5°F, blood pressure of 108 systolic, 76 diastolic, and a pulse of 110 per min.

TABLE 2
Immunoglobulin response in leptospirosis

Patient No.	Day of illness	Blood				Cerebrospinal fluid			
		IgG (mg%)	IgA (mg%)	IgM (mg%)	C'3 (mg%)	IgG (mg%)	IgA (mg%)	IgM (mg%)	C'3 (mg%)
	3	900	157	53	172	4.4	3.0	0	0
1	12	1,230	220	181	233	7.8	4.4	7.9	0
	21	1,455	338	170	203	—	—	—	—
	2	1,270	203	51	175	2.3	0	0	0
2	11	1,250	230	282	128	5.2	0	0	0
	20	1,500	229	115	202	—	—	—	—
	2	1,223	298	92	176	3.6	0	0	0
3	11	920	180	104	194	4.9	0	3.5	0
	20	1,150	185	330	170	—	—	—	—

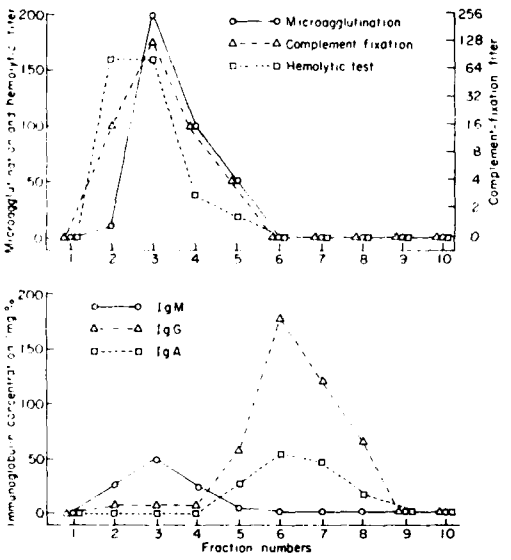

FIGURE 1. Sucrose density-gradient ultracentrifugation of sera obtained on the 11th day of illness from patient No. 1 who was infected with *Leptospira autumnalis*.

There was bilateral conjunctival injection and severe tenderness of the lumbar, gastrocnemic, and pectoral muscles on palpation.

Laboratory examination revealed a hematocrit of 36%, a WBC count of 6,850 per mm^3 with 87% neutrophils, corrected erythrocyte sedimentation rate of 34 mm per hour, and a platelet count of 90,000 mm^3. The total bilirubin was 0.9 mg%, SGPT 30 units, SGOT 130 units, and alkaline phosphatase 6 Bodansky units. Total serum protein was 5.3 g, albumin 2.5 g, alpha$_1$ globulin 0.28 g, alpha$_2$ globulin 0.68 g, beta globulin 0.56 g, and gamma globulin 1.26 g per 100 ml. The blood urea nitrogen was 31 mg and serum creatinine 3.0 mg per 100 ml. Spinal fluid examination revealed values for protein of 29 mg and for glucose of 73 mg per 100 ml; no cells were seen. The urine contained 1+ albumin. Twenty-four hour creatinine clearances on the 3rd and 4th days of illness were 20 and 10 ml per minute respectively. Microagglutination, complement-fixation, and hemolytic tests for leptospirosis were negative on admission (Table 1).

Because of the severe myalgia, meningismus, and fever, leptospirosis was strongly suspected, and the patient was treated with 10 million units of intravenous penicillin per day. Twelve hours after the start of therapy, his symptoms rapidly improved. On the 7th day of illness, the patient noticed areas of hypesthesia over the dorsum of his right hand and forearm and was unable to dorsiflex his right wrist. He was diagnosed as having a palsy of the right radial and ulnar nerves.

Repeat laboratory studies on the 11th day of illness revealed a hematocrit of 36%, WBC count of 6,600 per mm^3 with a normal differential count, and an erythrocyte sedimentation rate of 3 mm per hour. The platelet count was 60,000 mm^3, SGPT 70 units, SGOT 90 units, BUN 64 mg, and serum creatinine 3.5 mg per 100 ml. A repeat spinal fluid examination was normal. At this time, microagglutination, complement-fixation, and hemolytic titers for *Leptospira pyrogenes* were all positive (Table 1). *L. pyrogenes* was subsequently cultured on Fletcher's medium from blood obtained the day of admission. The immunoglobulin response during the septicemic and immune phases is shown in Table 2. A four-fold rise in the blood level of IgM and slight increases in IgG and IgA were noted the 11th day. The concentration of IgG in the spinal fluid also increased from 2.3 mg to 5.2 mg per 100 ml. Before discharge from the hospital, the BUN had dropped to 13 mg and the creatinine to 1.3 mg per 100 ml. The patient's right wrist drop and areas of hypesthesia over his right hand and forearms persisted for 3 months.

Case 3

A 19-year-old man entered the hospital with a 1-day history of diffuse arthralgia, fever, chills, headache, and photophobia.

Physical examination revealed a temperature of 104°F, blood pressure 176 systolic, 80 diastolic, and pulse of 130 per minute. There was bilateral conjunctival suffusion and generalized muscle tenderness on palpation.

Initial laboratory studies showed a hematocrit of 40%, a WBC count of 18,300 mm^3 with 91% polymorphonuclear neutrophils, and a corrected erythrocyte sedimentation rate of 24 mm per hour. The total bilirubin was 0.4 mg%, SGOT 14 units, SGPT 19 units, alkaline phosphatase 6 Bodansky units, and BUN 18 mg%. The serum protein was 7.2 g, albumin 3.95 g, alpha$_1$ globulin 0.33 g, alpha$_2$ globulin 1.04 g, beta globulin 0.84 g, and gamma globulin 1.04 g per 100 ml. Spinal fluid examination was normal, and the micro-

agglutination, complement-fixation, and hemolytic tests for leptospirosis were negative on admission.

The patient continued to have temperature elevations to 101° to 104°F until the 6th day of illness. His symptoms then gradually subsided, and he was clinically well by the 10th day. Laboratory tests at this time showed a hematocrit of 39%, a WBC count of 9,800 mm³ with 46% polymorphonuclear neutrophils, and an erythrocyte sedimentation rate of 25 mm per hour. The remainder of the laboratory tests were normal. A repeat spinal fluid examination revealed 26 neutrophils and 4 lymphocytes. The serum microagglutination test was positive at 1:1,600 for *Leptospira bataviae*; the titer in the CSF was 1:200. Results of complement-fixation and hemolytic tests are shown in Table 1. The immunoglobulin response during the acute phase of infection is shown in Table 2. A rise in the IgM fraction was again noted on the 11th day of illness.

DISCUSSION

Improvement of clinical symptoms in three patients with leptospirosis was closely associated with the appearance of circulating agglutinins as well as complement-fixing and hemolytic antibodies. Twofold to fourfold increases in IgM and rises in the levels of IgA and IgG in the sera were also seen. Ultracentrifugation studies showed that IgM was the predominant immunoglobulin involved in the agglutination, complement-fixation, and hemolytic reactions. These results confirmed earlier studies that demonstrated that a major portion of agglutinating and hemolytic antibodies for seven serotypes of leptospira was 19S macroglobulins, which were still detectable in high titers 4 to 8 weeks after the onset of symptoms.[10]

The recurrence of meningitis during the immune phase of this disease has been attributed to an immunological response involving antigen and antibody rather than to direct injury to the meninges by leptospira or its products. Invasion of the subarachnoid space by leptospira occurs early in the course of illness, but no inflammatory response is elicited by its presence. As immunity develops, clearance of the invading organisms from the CSF is associated with the appearance of pleocytosis. The transient signs of meningitis at this time may be due to the reaction of antibodies with leptospiral antigens.[3] In patient Number 1, *L. autumnalis* was recovered from an otherwise normal CSF the 3rd day of illness. With the recurrence of fever and meningitis on the 11th day, strain-specific agglutinins, an increase in leukocytes, and leptospira were detected in the spinal fluid. Increases in concentration of IgG and IgA and the appearance of IgM in the CSF were also noted, and it is conceivable that an inflammatory reaction was elicited by leptospira-antibody complexes in the central nervous system.

Although they are infrequent, neurological complications such as peripheral and cranial nerve palsies, radiculitis, transverse myelitis, and encephalomyelitis occur during the immune phase and may require weeks to months for complete resolution. Peripheral nerve lesions also appear during the septicemic phase but are less serious and usually subside with clearing of the leptospiremia. Lesions that are seen during the immune phase are similar to the neurological sequelae of serum sickness and may also be manifestations of an antigen-antibody reaction.[4] The radial and ulnar nerve palsy that appeared at onset of the immune phase in patient Number 2 required a period of 3 months before improvement was noted.

The pathogenesis of fever during the septicemic phase has been attributed to a leptospiremia. However, the temperature rise during the immune phase occurs at a time when antibody titers are rising and leptospira are being destroyed, and it has been suggested that this recurrence of fever may be the result of a hypersensitivity reaction to leptospiral antigens.[3]

The development of immunity in leptospirosis requires the production of strain-specific antibodies. The exact role of the antibodies has not been clarified, but their presence appears to enhance phagocytosis by opsonization and agglutination of the infecting agents.[11] Sufficient levels of antibodies are apparently needed for clearing of leptospira since these agents may persist in tissues with low antibody titers, such as the glomerular filtrate and the aqueous humor of the eye.[3]

The role of antibodies in the pathogenesis of the immune phase is less clear. Agglutination of leptospiral organisms plays more of a protective role and facilitates phagocytosis. However, since IgM complement-fixing antibodies are also present, one possible pathogenetic mechanism may be activation of the complement system by anti-

gen-antibody complexes, which in turn may cause tissue damage and give rise to the clinical symptoms that are seen. Additional studies are needed to clarify the pathogenetic mechanisms responsible for the immune phase of leptospirosis.

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