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Chemotaxis of Leptospires to Hemoglobin in Relation to Virulence

KAZUYO YURI, Y. TAKAMOTO, MAYUMI OKADA, T. HIRAMUNE,*
N. KIKUCHI, AND R. YANAGAWA

Department of Epizootiology, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069, Japan

Received 26 October 1992/Accepted 19 February 1993

A guinea pig-lethal line of *Leptospira interrogans* serovar *copenhageni* strain Shibaura, but not an avirulent line of the same strain, moved in larger numbers toward hemoglobin than toward distilled water (control) in a U-shaped polypropylene tube. *L. interrogans* serovar *lai* strains 017 and KH-1, which were also guinea pig lethal, showed a similar move to hemoglobin. No such move toward hemoglobin was shown by 14 avirulent strains of *L. interrogans* (with one exception) or any of the 8 strains of *L. biflexa* tested.

Leptospires grow in surface water or wet soil and infect humans and animals, particularly when humans and animals have cuts and skin abrasions (3). It is not known whether leptospires approach skin randomly or by a specific route. An investigation in our laboratory with a virulent strain of *Leptospira interrogans* serovar *copenhageni* and semisolid Korthof's medium in a U-shaped polypropylene tube showed chemotaxis of leptospires toward hemoglobin (4). Takamoto, of our laboratory (5a), found that no chemotaxis of leptospires toward hemoglobin occurred when the strain was not sufficiently virulent. The purpose of the present investigation was to study chemotaxis of leptospires toward hemoglobin in relationship to virulence by using virulent and avirulent strains of pathogenic leptospires and strains of saprophytic leptospires.

In the present study, we used 4 virulent and 14 avirulent strains of *L. interrogans*, 8 strains of *L. biflexa*, and 2 strains of *Leptonema illini* (Table 1). Of the virulent strains, *L. interrogans* serovar *copenhageni* strain Shibaura line 1 and *L. interrogans* serovar *lai* strains 017 and KH-1 were guinea pig (180 g) and hamster (50 g) lethal and *L. interrogans* serovar *canicola* strain Moulton was hamster lethal. *L. interrogans* serovar *lai* was a main causal agent of a recent leptospirosis epidemic in The People's Republic of China (2). The virulence of these strains was maintained by intraperitoneal passage through guinea pigs or hamsters. Animal experiments were conducted as previously described (3). Only the first subculture of a blood culture of the virulent strains from a moribund guinea pig or hamster was used in the chemotaxis experiments. The remaining strains of *L. interrogans*, which had been passed through media for at least 38 years, were avirulent. *L. interrogans* serovar *copenhageni* strain Shibaura line 5 was an avirulent derivative of Shibaura line 1 and has been avirulent for more than 21 years (6).

The strains were cultured in Korthof's medium (5) to which 10% rabbit serum and a small amount of phenol red (as a pH indicator) were added. Incubation of the cultures was done for 3 to 10 days at 30°C. The medium used for the chemotaxis experiments is described below.

Chemotaxis of leptospires toward hemoglobin was studied as described previously, with semisolid Korthof's medium in U-shaped polypropylene tubes (4) (Fig. 1). The following modifications were made. (i) Commercially available straw-shaped, opaque polypropylene tubes (flexible straw) measuring 5 mm in diameter (Glico Co., Osaka, Japan) were used to prepare the U-shaped tubes. (ii) Semisolid Korthof's medium was semisolidified with 0.1% agar (Noble agar; Difco, Detroit, Mich.) as described by Takamoto (5a). The total length of the medium was 20 mm (volume, 0.35 ml). The U-shaped tubes were placed in a rack and covered with aluminum foil. (iii) The volume of the leptospiral inoculum was 30 μl. Leptospires were gently inoculated onto the top of the medium of one side of each U-shaped tube, while the top of the other side of the tube received the same volume of (i) sterilized concentrations of hemoglobin or (ii) distilled water (control). Ten U-shaped tubes were used for each concentration of hemoglobin and for distilled water. After incubation at 30°C for 24 h, a specimen taken with a platinum loop from the top of the medium on the uninoculated side was examined under a dark-field microscope and numbers of migrated leptospires per field were counted at a magnification of ×200 (ocular, 10×; objective, 20×). Average numbers of migrated leptospires per 10 U-shaped tubes were compared between uninoculated tops containing hemoglobin and distilled water. A strain was determined to be chemotactic toward hemoglobin if the numbers of leptospires that moved toward the uninoculated tops with hemoglobin were significantly larger than the numbers of leptospires that moved toward the uninoculated tops containing distilled water (control).

Student's *t* test was used to determine the difference between the numbers of leptospires that moved to the top of the medium on the uninoculated side in U-shaped tubes containing either various amounts of hemoglobin or distilled water. The χ^2 test was used to determine the difference between the percentages of U-shaped tubes in which leptospires moved to the top of the medium on the uninoculated side in tubes containing either various amounts of hemoglobin or distilled water.

Bovine hemoglobin was prepared as previously described (4). One volume of bovine erythrocytes, thoroughly washed with phosphate-buffered saline, was lysed with 9 volumes of sterilized distilled water at 4°C overnight. The hemoglobin content of the preparation, calculated by the cyan-methemoglobin method (1), was 210 μg/30 μl. The preparation was

* Corresponding author.

TABLE 1. Chemotaxis of leptospires toward hemoglobin in relationship to virulence

Species and serovar	Strain	No. of leptospires that moved toward uninoculated tops covered with the following concn of hemoglobin (μg/30 μl)[a]:				Animal(s) for which strain is lethal
		21	2.1	0.21	0 (control)	
L. interrogans						
copenhageni	Shibaura virulent line 1	20.5	53.5	113.5	0.9	Guinea pig, hamster
lai	017	16.6	30.2	5.2	0.5	Guinea pig, hamster
lai	KH-1	20.9	37.2	5.5	0.4	Guinea pig, hamster
canicola	Moulton	1.7	2.7	2.8	0.4	Hamster
copenhageni	Shibaura avirulent line 5	0.1	0	0.5	0.5	—[b]
copenhageni	M20	0	0	0	0	—
icterohaemorrahagiae	Ictero no. 1	0.5	0.4	0.6	0.1	—
icterohaemorrhagiae	RGA	0.5	0	0	0	—
australis	Ballico	0.3	0	0	0.4	—
autumnalis	Akiyami A	0	0	0	0	—
canicola	Hond Utrecht IV	0	0	0	0	—
grippotyphosa	Moskva V	0.1	0.3	0	0.6	—
hardjo	Hardjoprajitno	0.5	0	0	0	—
hebdomadis	Hebdomadis	0.7	3.6	5.7	1.0	—
javanica	Veldrat Batavia 46	0	0.1	0	0	—
pomona	Pomona	0.4	0	0	0.7	—
pyrogenes	Salinem	0	0.3	0	0.2	—
tarassovi	Mitis Johnson	0	0.2	0.2	0.1	—
L. biflexa						
andamana	CH11	0	0	0	0	
holland	Waz Holland	0	0.1	0.2	0.2	
patoc	Patoc I	0	0.1	0	0	
Untyped	965	0	0	0	0	
Untyped	LT43	0	0	0	0	
Untyped	Gent	0	0	0	0	
Untyped	Urawa	0	0.2	0	0	
Untyped	A284	0	0	0	0	
L. illini	3055	0	0	0	0	
	Dimbo	0	0.5	0	0	

[a] Number of leptospires per field (magnification, ×200); average per 10 tubes. Typical results of repeated experiments are shown (see text). Underlined values are significantly larger than control (Student's *t* test).

[b] —, passed through media for at least 38 years and no longer virulent. Shibaura avirulent line has been avirulent for more than 21 years.

diluted with distilled water, and 21, 2.1, or 0.21 μg of hemoglobin per 30 μl was used.

Table 1 shows typical results of the experiments, which were repeated three times. The experiments in which leptospires moved toward hemoglobin in significantly larger numbers than those that moved toward distilled water were repeated six times.

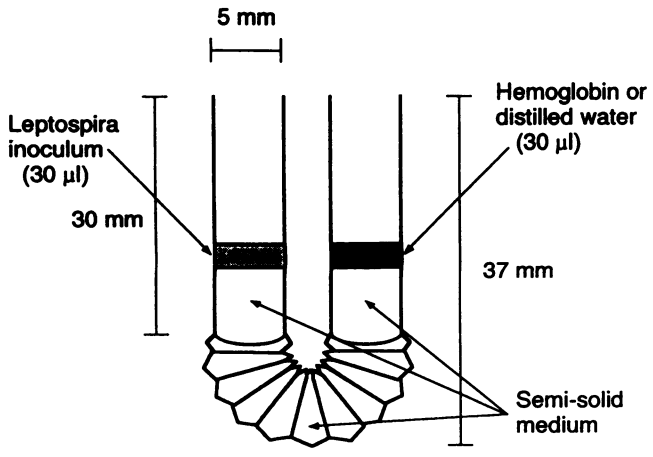

FIG. 1. Diagram of a U-shaped polypropylene tube made of a commercially available flexible straw.

Of the virulent strains of *L. interrogans*, which are lethal to guinea pigs and hamsters and belong to serogroup Icterohaemorrhagiae, *L. interrogans* serovar *copenhageni* strain Shibaura virulent line moved toward the uninoculated tops containing 21, 2.1, and 0.21 μg of hemoglobin; the numbers of leptospires that moved were 20.5, 53.5, and 113.5 per field, respectively. The numbers of leptospires that moved toward the uninoculated tops containing hemoglobin were significantly larger than the number of leptospires that moved toward the uninoculated tops containing distilled water (control), which was 0.9 per field. Similarly, *L. interrogans* serovar *lai* strains 017 and KH-1 showed a significant move toward hemoglobin. The results were similar in repeated experiments.

L. interrogans serovar *canicola* strain Moulton, lethal to hamsters but not to guinea pigs, showed a significant move toward hemoglobin, but in only two of six experiments. Moreover, the numbers of leptospires of *L. interrogans* serovar *canicola* strain Moulton that moved toward hemoglobin were considerably smaller than those of guinea pig-lethal *L. interrogans* serovar *copenhageni* strains Shibaura line 1 and *L. interrogans* serovar *lai* strains 017 and KH-1. In

the remaining four experiments, the numbers of leptospires that moved toward hemoglobin were very small and not significantly different from those of leptospires that moved toward distilled water (data not shown).

Avirulent strains of *L. interrogans*, in contrast, did not show a significant move toward hemoglobin. In these strains, the numbers of leptospires that moved toward hemoglobin were very small and not significantly different from those of leptospires that moved toward distilled water. The avirulent line (line 5) of *L. interrogans* serovar *copenhageni* strain Shibaura was among the avirulent strains. The results were similar in repeated experiments. The only exception was *L. interrogans* serovar *hebdomadis* strain Hebdomadis, which did show a significant move toward hemoglobin. The numbers of leptospires of this strain that moved toward hemoglobin were considerably smaller than those of the guinea pig-lethal *L. interrogans* serovar *copenhageni* strain Shibaura line 1 and *L. interrogans* serovar *lai* strains 017 and KH-1. *L. interrogans* serovar *hebdomadis* strain Hebdomadis, however, showed such a move to hemoglobin in four of six experiments.

In all of the above-described experiments, the significant differences shown by Student's t test ($P < 0.01$) were also significant by the χ^2 test ($P < 0.05$).

None of the saprophytic strains, eight strains of *L. biflexa*, and two strains of *Leptonema illini* showed a significant move toward hemoglobin. Repeated experiments gave similar results, without exception.

No chemotaxis of the guinea pig-lethal strains of leptospires was shown when the medium was deprived of rabbit serum. Culture media other than Korthof's were not used.

The above-described experiments indicated that guinea pig-lethal strains of *L. interrogans* showed chemotaxis toward hemoglobin, while avirulent strains of *L. interrogans*, with one exception, and saprophytic leptospires (*L. biflexa* and *L. illini*) did not show such chemotaxis. These results lead to the hypothesis that virulent leptospires can recognize hemoglobin in surface water, which is the most common habitat of leptospires, reach cuts and abrasions on the skin (perhaps ascending a gradient of hemoglobin), and invade the bodies of humans and animals. This hypothesis may be useful in further investigations into the initiation of natural infection with leptospires.

Virulence of *L. interrogans* has been known to be lost by passages through media. Chemotaxis toward hemoglobin may also be demonstrated primarily by virulent leptospires and lost after passages through media. Whether plasmids are associated with leptospiral virulence and chemotaxis toward hemoglobin is under investigation, together with chemotaxis of leptospires toward fatty acids.

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