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Proteolytic Action of *Legionella pneumophila* on Human Serum Proteins

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Proteolysis by four strains of *Legionella pneumophila*, the etiological agent of Legionnaires disease, was studied by the method of immunoelectrophoresis. Twenty-three human serum proteins were tested as substrates. Five proteins were degraded: α_1-acid glycoprotein, α_1-antichymotrypsin, β-lipoprotein, β_{1E}-globulin, and β_2-glycoprotein-I. Moreover, the degradation of α_1-antichymotrypsin was demonstrated by investigation of an enzyme-blocking test. It is suggested that the proteolytic activity of *L. pneumophila* may bear some relationship to its pathogenic activity.

Previous studies from our laboratory described immunoelectrophoresis as a sensitive procedure to detect proteolytic activities of microorganisms (6-12, 14-16). Since the cause of pulmonary and extrapulmonary disturbances in Legionnaires disease is not known and an exotoxin of *Legionella pneumophila* was recently described (1), we used this technique for the examination of proteolytic activity. The results of this *L. pneumophila* study are the subject of this report.

MATERIALS AND METHODS

Bacteria. Four strains of *L. pneumophila* designated Philadelphia 2, Togus, Bloomington, and Los Angeles, serogroups 1, 2, 3, and 4, respectively, were kindly provided by G. Wewalka, Wien, Austria. He received the strains from the Center for Disease Control, Atlanta, Ga. Mueller-Hinton medium supplemented with 5% sheep blood and 1% (vol/vol) IsoVitaleX (BBL Microbiology Systems) enrichment (5) and an appropriate charcoal agar (2) were used for maintaining the strains and for inoculum cultures for the immunoelectrophoresis and the enzymatic test. Agar plates were incubated at 35°C in an atmosphere of air plus 2.5% carbon dioxide (2).

Immunoelectrophoresis procedure. Cells were harvested after 3 days in 0.1 M phosphate-buffered saline, pH 7.0, and shaken to form a homogeneous suspension which was used for immunoelectrophoresis and then also for checking their ability to destroy the blocking action of α_1-antichymotrypsin. The preparation had a cell content of 50% (vol/vol) as determined by bacteriocrit. For testing proteolytic activity, 0.5 ml of the bacterial suspension was incubated with 0.5 ml of normal human serum at 37°C for 4 h. Serum samples diluted with phosphate-buffered saline (1:1), but without bacteria, and serum samples with a bacterial suspension heated at 95°C for 30 min served as negative controls. The immunoelectrophoreses were performed by the micromodification of Scheidegger (13). The agar was prepared by dissolving 2 g of pure agar (Behringwerke AG, Marburg, West Germany) in 100 ml of diethylbarbiturate acetate buffer, pH 8.2 (μ = 0.05). Quantities (3 ml each) of the hot agar solution were pipetted onto precleaned glass slides (76 by 26 mm). The agar wells were cut by means of a template. The buffer used was a Michaelis diethylbarbiturate acetate buffer, pH 8.2 (μ = 0.1). The electrophoretic separation of the serum was achieved within 180 min. The voltage was 1 V/cm. The monospecific rabbit antisera against 23 different human serum proteins which were used in this study were obtained from Behringwerke AG, Marburg, West Germany, and are listed in Table 1.

α_1-Antichymotrypsin activity tests. The prote-

TABLE 1. *Proteolytic actions of L. pneumophila on human serum proteins demonstrated by immunoelectrophoresis*

Expt no.	Tested protein	Result
1	Prealbumin	-
2	Albumin	-
3	α_1-Lipoprotein	-
4	α_1-Acid glycoprotein	+ (Fig. 1)
5	α_1-Antitrypsin	-
6	α_1-Antichymotrypsin	+ (Fig. 2)
7	α_1-β-Glycoprotein	-
8	Inter-α-trypsin inhibitor	-
9	Haptoglobin	-
10	Ceruloplasmin	-
11	Gc-globulin	-
12	α_2-Macroglobulin	-
13	α_2-HS-glycoprotein	-
14	α_2-Zn-glycoprotein	-
15	β-Lipoprotein	+ (Fig. 3)
16	Transferrin	-
17	β_{1C}/β_{1A}-Globulin	-
18	β_{1E}-Globulin	+ (Fig. 4)
19	Hemopexin	-
20	β_2-Glycoprotein-I	+ (Fig. 5)
21	IgA[a]	-
22	IgM	-
23	IgG	-

[a] IgA, Immunoglobulin A.

olytic destruction of α_1-antichymotrypsin by *L. pneumophila* was tested by measurements of the activity of chymotrypsin in fresh human feces solution as an available source of human chymotrypsin and its inhibition by human serum α_1-antichymotrypsin, respectively. For assay of chymotrypsin activity, the liberation of *p*-nitroaniline from *N*-succinyl-L-phenylalanine-*p*-nitroanilide was colorimetrically determined as described by Fritz et al. (3).

RESULTS AND DISCUSSION

The results shown in Table 1 and Fig. 1 to 5 indicate that *L. pneumophila* degrades 5 of 23 serum proteins, i.e., α_1-acid glycoprotein (orosomucoid), α_1-antichymotrypsin, β-lipoprotein, β_{1E}-globulin, and β_2-glycoprotein-I. α_1-Acid glycoprotein and also β_{1E}-globulin show slight cathodic shifts of their bands (Fig. 1 and 4). α_1-Antichymotrypsin is degraded, and the remaining band is also shifted to the cathode (Fig. 2). Degradation of β-lipoprotein is compared by an anodic shift of the remaining precipitate (Fig. 3), and finally β_2-glycoprotein-I is degraded without a visible shift.

To show the biological inactivation of serum α_1-antichymotrypsin by *L. pneumophila*, the enzymatic activity of human chymotrypsin in normal feces was measured without and with the addition of serum and with the addition of serum preincubated with *L. pneumophila*. The results in Table 2 show that the activity of chymotrypsin in the feces was 6.0 IU/liter, or 100% (experiment 1). Serum and *L. pneumophila* suspension had no detectable activities (experiments 2 and 3). Most of the chymotrypsin activity was blocked by addition of homologous human serum (experiment 4). Preincubation of the serum

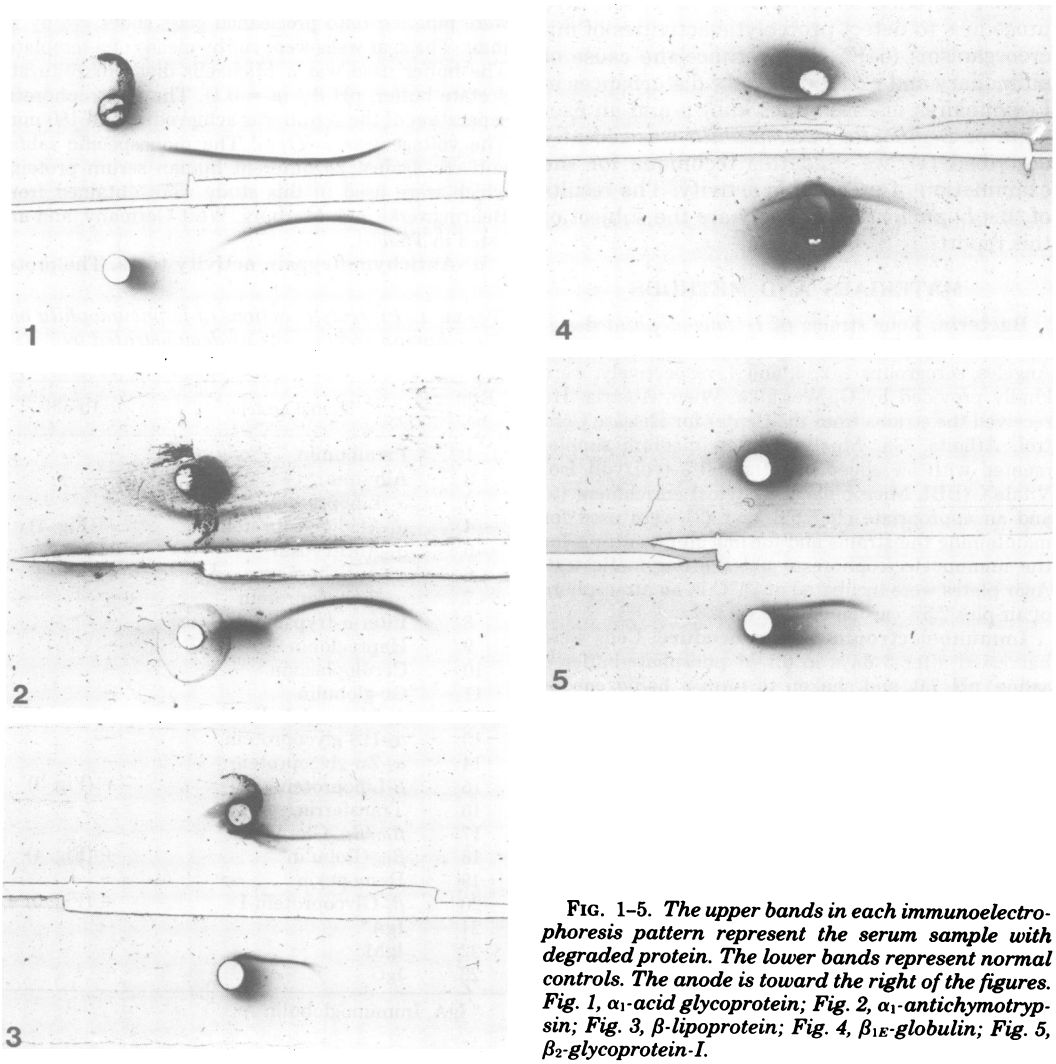

FIG. 1–5. *The upper bands in each immunoelectrophoresis pattern represent the serum sample with degraded protein. The lower bands represent normal controls. The anode is toward the right of the figures. Fig. 1, α_1-acid glycoprotein; Fig. 2, α_1-antichymotrypsin; Fig. 3, β-lipoprotein; Fig. 4, β_{1E}-globulin; Fig. 5, β_2-glycoprotein-I.*

TABLE 2. *Enzymatic activity of chymotrypsin, blocking function of α_1-antichymotrypsin, and destroying action of L. pneumophila on the blocking function of α_1-antichymotrypsin*

Expt no.	Reaction mixture and procedure[a]	Chymotrypsin activity	
		IU/liter	%
1	0.1 ml of feces[b]–0.2 ml of saline	6.0 ± 0.2	100 ± 3
2	0.1 ml of serum[c]–0.2 ml of saline	0	0
3	0.1 ml of bacteria[d]–0.2 ml of saline	0	0
4	0.1 ml of feces–0.1 ml of serum–0.1 ml of saline	1.0 ± 0.2	17 ± 3
5	0.1 ml of serum–0.1 ml of bacteria, 4-h incubation at 37°C, + 0.1 ml of feces	3.9 ± 0.2	65 ± 3

[a] After mixture of the components, buffer and substrate were added, and the colorimetric enzyme reaction was performed as described elsewhere (3).

[b] Human feces suspended in saline and centrifuged, 10% (vol/vol).

[c] Human serum.

[d] Suspension of *L. pneumophila* in phosphate-buffered saline, 50% (vol/vol).

with *L. pneumophila*, however, destroyed most of the blocking activity of the serum, i.e., the α_1-antichymotrypsin activity was omitted and the chymotrypsin activity increased (experiment 5). A control shows that heating destroys the ability of *L. pneumophila* to destroy the α_1-antichymotrypsin.

Comparison of the patterns of the Fig. 1 to 5 suggests that the degradation of serum proteins by *L. pneumophila* is due to several (at least two) different enzyme systems. β-Lipoprotein is degraded with an anodic shift of its remaining precipitate; β_2-glycoprotein-I is degraded without a detectable shift; and α_1-acid glycoprotein, α_1-antichymotrypsin, and β_{1E}-globulin are degraded with more or less cathodic shifts. The molecular pathomechanism of *L. pneumophila* may involve proteolytic enzymes, as was suggested by Baine et al., who demonstrated an exotoxin activity of *L. pneumophila* by showing hemolysis with guinea pig cells (1).

The inactivation of the α_1-antichymotrypsin by *L. pneumophila* may cause a pulmonary syndrome possibly in a manner comparable to the chronic pulmonary insufficiency caused by an antitrypsin deficiency (4).

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