An Experimental Model of Pseudotuberculosis Infection in Monkeys

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An experimental model of pseudotuberculosis infection was developed using *Papio hamadryas* monkeys. Clinical manifestations and some microbiological and immunological aspects of pseudotuberculosis caused by oral infection of animals are studied. The values of LD_{50} of the pseudotuberculosis agent are established for monkeys, white mice, and guinea pigs. Oral infection of monkeys with sublethal doses of *Yersinia pseudotuberculosis* is found to induce a pronounced specific immunity. The efficacy of immunoblotting for serologic diagnosis of pseudotuberculosis in clinically obscure cases is demonstrated.

Key Words: *pseudotuberculosis; monkeys; oral infection; immunoblotting*

The problem of pseudotuberculosis is attracting the attention of specialists more and more of late. One of the important research trends is the development of methods for emergency and specific prevention and treatment of the disease [2,4,6]. Experimental simulation of this infection is usually carried out by parenteral inoculation of small laboratory animals, such as mice and guinea pigs [8,9]. The few publications on the course of pseudotuberculosis in monkeys [6] are empirical and do not permit an unambiguous judgment on the possibility of using these animals for the reproduction of pseudotuberculosis in experimental biomedical investigations.

The present research was aimed at investigating the clinical manifestations and microbiological and immunological aspects of experimental pseudotuberculosis in monkeys infected orally, i.e., via the route natural for this infection.

MATERIALS AND METHODS

The Ca-dependent strain 147 of *Y. pseudotuberculosis* serotype I, containing a plasmid with a molecular weight of 47 MD, was used in the experiments with monkeys. The cultures were grown at 4°C for 5 days in Hottinger agar, pH 7.2. In immunoblotting cultures of *Y. pseudotuberculosis* strain 147 were used, as well as of *Y. enterocolitica* strain 657 (serotype 0:9) grown for 2 days at 37°C in the same medium with $CaCl_2$ added until a 1 mmol concentration was attained. Outbred white mice weighing 18-20 g, guinea pigs weighing 300-350 g, and *Papio hamadryas* monkeys weighing 4-6 kg were used. The animals were infected orally, via a syringe fitted with an "olive" needle, with a bacterial suspension in 10% $NaHCO_3$ solution. Before infection the animals were kept fasting for 24 h. The follow-up period in all the experiments was 21 days. Individual monkeys' sera were tested in immunoblotting. Electrophoresis of bacterial culture lysates was carried out in polyacrylamide gel (linear gradient from 5 to 15%) with sodium dodecyl sulfate according to a previously described technique [7] using a PROTEAN II xi device (Bio-Rad, USA). Immunoblotting was carried out as described previously [10] using a Transphor TE50 device (Hoefer, USA). Rectal temperature was measured daily in monkeys. Before infection and

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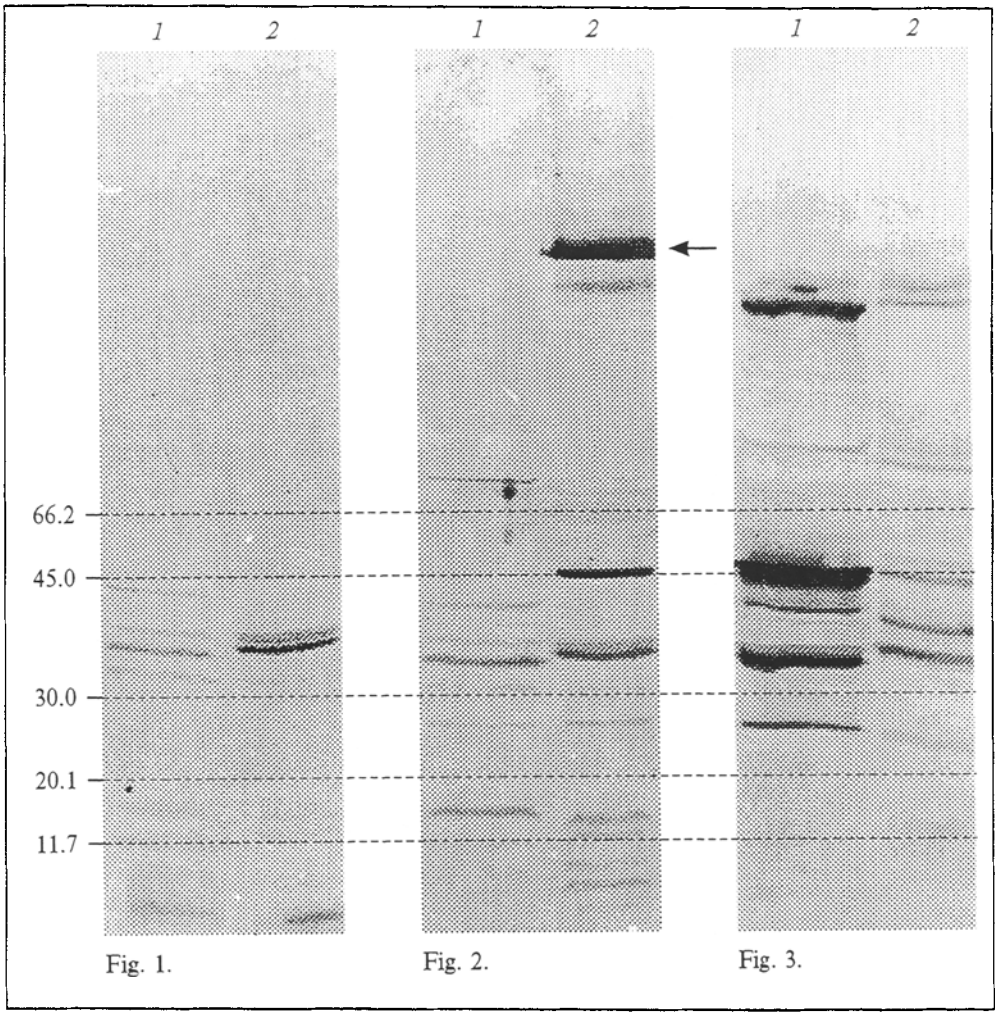

Fig. 1. Fig. 2. Fig. 3.

Fig. 1. Immunoblot of serum from intact monkey № 1. Here and in Figs. 2 and 3: preparations of lysed bacterial cultures are applied to strips *1* and *2*: *1*) *Y. pseudotuberculosis*, strain 147; *2*) *Y. enterocolitica*, strain 657, both strains grown at 37°C. At left of immunoblots: molecular weight of marker proteins.

Fig. 2. Immunoblot of serum from intact monkey № 10.

Fig. 3. Immunoblot of serum from monkey № 6 orally infected with a culture of *Y. pseudotuberculosis* strain 147.

on days 2, 7, and 10 after it oral smears were collected and inoculated in Serov's medium [5]. Fecal samples from monkeys were inoculated daily in Serov's medium over the whole follow-up period. In dead animals the pathomorphological picture of visceral changes was examined and samples of blood, homogenates of lungs, liver, spleen, and lymph nodes, and of intestinal contents were inoculated in Serov's medium. The LD_{50} values and confidence intervals with a 95% probability were estimated from guidelines [1].

RESULTS

Table 1 presents the results of primary infection of monkeys. The duration of the incubation period depended on the infective dose. In monkeys Nos. 7 and 8 the incubation period lasted for 3 days. However, after infection with higher doses (monkeys Nos. 9-12) the disease developed as soon as 24 h postinoculation. The same monkeys developed a short-term (1 day) fever (>39.5°C). The disease in monkeys started with symptoms of gastroenteritis: loose stool with blood 3-4 times a day, vomiting, general weakness, limpness, lethargy, refusal to eat, photophobia. All the monkeys falling ill presented with signs of catarrhal inflammation of the upper respiratory tract during the initial period and, at the peak of the disease, rhinitis and coughing. Hyperemia of the conjunctiva and injection of scleral vessels and puffiness and hyperemia of the muzzle and neck were observed at the peak of the disease. Besides the said symptoms, monkeys Nos. 9-12 developed on days 1-2 a punctate rash localized in the armpits and around the groin. Directly before death the temperature fell to 37.5-38.0°C. Dead monkeys Nos. 9, 11, and 12 had foam at the mouth. A 1-1.5 kg loss of body weight was recorded in all monkeys which died. Autopsy revealed vast zones of erosion and ulcer of the intestinal mucosa. The lymph nodes of the small intestine mesentery were involved: they were enlarged to 1-3 cm in diameter, colored grayish-pinkish, with a solid consistency. The serous coating of the distal portion of the small intestine and cecum was edematous, with dilated vessels; in monkeys Nos. 9, 11, and 12 it exhib-

TABLE 1. Results of Primary Oral Infection of Monkeys with *Y. pseudotuberculosis*

№ of animal	Infective dose, live bacteria	Maximal rectal temperature, °C	Symptoms of gastrointestinal involvement	Isolation of agent from feces	Outcome of infection
1	4.2×10^5	38.6	Absent	Not isolated	Survived
2		38.6	— " —	— " —	— " —
3	4.2×10^6	38.9	— " —	— " —	— " —
4		39.0	— " —	— " —	— " —
5	4.2×10^7	39.4	— " —	— " —	— " —
6		39.3	— " —	— " —	— " —
7	4.2×10^8	39.4	Gastroenteritis, loose stool with blood	Isolated	— " —
8		39.4	— " —	— " —	Died on day 6
9	4.2×10^9	39.8	— " —	— " —	Died on day 3
10		39.7	— " —	— " —	Died on day 5
11	4.2×10^{10}	39.9	— " —	— " —	Died on day 3
12		39.8	— " —	— " —	Died on day 4

ited hemorrhages and fibrinous incrustation. Up to 40-80 ml of serous and serous-hemorrhagic exudation was found in the abdominal cavity of dead monkeys. Granulomas and microabscesses in the liver were found in monkeys Nos. 8 and 10.

In none of the cases were bacteria isolated from the blood or lungs of dead monkeys. On the other hand, *Y. pseudotuberculosis* were isolated from the intestinal contents, lymph nodes of the ileocecal area, and liver by inoculation in Serov's medium. *Y. pseudotuberculosis* cells were never detected in oral smears, while from feces the agent culture was isolated from monkeys Nos. 7-12.

Our findings permitted us estimate the LD_{50} value of strain 147 for monkeys, equal to 8.7×10^8:2.3 live bacteria. The susceptibility of small laboratory animals to pseudotuberculosis was found to be much higher. The LD_{50} values of the same strain for white mice and guinea pigs infected orally were 8.5×10^4:2.2 and 6.9×10^6:2.7 live bacteria, respectively.

Our estimated LD_{50} of *Y. pseudotuberculosis* for monkeys is compatible with the value of the minimal infective dose (0.9×10^9 bacteria) which, taken orally, induced disease in a well-known experiment with autoinfection performed by V. A. Znamenskii [3]. The clinical manifestations of pseudotuberculosis in monkeys which we observed correspond to the clinical picture of this disease in humans [6]. On the whole, the findings indicate evident advantages of monkeys as a model, in

TABLE 2. Results of Repeated Oral Infection of Monkeys with *Y. pseudotuberculosis*

№ of animal	Infective dose, live bacteria	Maximal rectal temperature, °C	Symptoms of gastrointestinal involvement	Isolation of agent from feces	Outcome of infection
1	1.2×10^9	40.0	Gastroenteritis, loose stool with mucus and blood	Isolated	Died on day 5
2	1.2×10^9	39.0	Loose stool for 2 days	Not isolated	Survived
3	1.2×10^9	39.0	Absent	Isolated	— " —
4	1.2×10^{10}	38.9	— " —	— " —	— " —
5	1.2×10^{10}	40.1	Gastroenteritis, loose stool with mucus	— " —	— " —
6	1.2×10^{10}	39.1	Loose stool for 3 days	— " —	— " —
7	1.2×10^9	38.9	— " —	— " —	— " —

comparison with mice and guinea pigs, for experimental reproduction of pseudotuberculosis.

On day 22 after primary infection a reinfection of surviving monkeys (Nos. 1-7) was carried out (Table 2). Only in two (Nos. 3 and 4) of the seven monkeys did we observe no symptoms of gastrointestinal involvement, even though the agent was isolated from feces. The disease ran a severe course in monkeys Nos. 1 and 5: high fever, manifest symptoms of intoxication, and gastrointestinal and upper respiratory involvement. Only monkey № 1 died; autopsy showed pathoanatomical changes characteristic of pseudotuberculosis.

Immunoblotting tests were carried out with the sera of all intact monkeys and survivors of both experiments. In all the animals except Nos. 3, 7, and 10 the spectra of antibodies in the sera collected before infection were identical and corresponded to the normal antibody spectrum of intact animals (Fig. 1). In monkeys Nos. 3, 7, and 10 antibodies to various antigens of *Y. enterocolitica* were found (Fig. 2). We may assume that these monkeys had intestinal yersiniasis during the period which preceded the experiment. Two of these three monkeys (Nos. 3 and 7) survived after primary infection with relatively low doses of *Y. pseudotuberculosis,* and monkey No.10 died, although it had antibodies to *Y. enterocolitica* protein YopA in the serum (Fig. 2, shown with an arrow).

Antibodies to *Y. pseudotuberculosis* antigen, including those to YopA protein, were detected in all monkeys surviving pseudotuberculosis 21 days after primary infection (Fig. 3). The increase of the humoral immune response to *Y. pseudotuberculosis* antigen in monkeys who had the disease was associated with a noticeable improvement of animal protection (Table 2). Hence, primary oral infection of monkeys with sublethal doses of *Y. pseudotuberculosis* is capable of conferring protective immunity. These results concur with the opinion of G. P. Somov *et al.* that a stable postinfection immunity develops in persons with a history of this infection [6].

Comparative analysis of immunoblotting tests of monkey No. 1 before and after primary infection indicated that the animal did not react to the primary infection and proved to be unprotected from reinfection (Table 2). According to published data [6], it is during the second and third weeks of the disease that the highest incidence of relapses is recorded, which is attributed to the slow development of specific immunity. Apparently, immunoblotting is to be regarded as an efficient method for serological diagnosis of pseudotuberculosis in clinically intricate and obscure cases when attempts at isolating the agent from the patient fail.

Hence, oral infection of monkeys is a good model for experimental simulation of pseudotuberculosis which may be used to assess the efficacy of means for the prevention and therapy of pseudotuberculosis, particularly so at the final stage of their trials.

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