EXPERIMENTAL BIOLOGICAL MODEL OF BACILLARY DYSENTERY

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The basic requirement for the successful pathogenic study of human infectious diseases is the experimental reproduction of the given disease in animals. E. V. Abramova (1938) first demonstrated that the course of bacillary dysentery was similar in monkeys and man. An experimental model of bacillary dysentery was recently perfected on monkeys at the Sukhumsk Medical-Biological Station of the Academy of Medical Sciences USSR, under the direction of V. L. Troitsky. During research on this problem, however, it was found that monkeys in captivity often incur the so-called "spontaneous" Flexner's dysentery, causing attempts to experimentally infect them with the Flexner's type of dysentery pathogens to fail.

We conducted experiments reproducing an experimental model of Flexner's dysentery on kittens. Our choice of this type of animal was based on the research of Grigoryev, who, as early as 1891, demonstrated the possibility of infecting kittens with bacillary dysentery pathogens, and also on the work of Abramova and Burova (Department of Parasitology, VIEM), which established that kittens in captivity incur bloody mucous dysentery, and, therefore, that pathogens of biological properties similar to the Grigoryev-Shiga, Flexner's and Sonnei strains can be obtained from them. By the time the work was completed, we had become acquainted with the data of E. A. Sergeevich (from the Molotov Institute of Vaccines and Sera), which, based on experiments conducted, indicated that the domestic cat could be used as an experimental-biological model for the reproduction of bacillary dysentery.

EXPERIMENTAL METHODS

The experiments were done on 16 kittens ranging in age from 2 to 5 months. The animals weighed from 510 to 1,280 grams. The kittens were previously quarantined and twice examined.

Bacteria of a local strain of Flexner's No. 6176, isolated from a dysentery patient on October 15, 1953, with typical morphological, cultural, biochemical and serological properties and an above average virulence, were used to infect the kittens. The infection was produced by a suspension of microbes in a physiological solution, obtained by washing from a 24-hour agar culture, 1-2-4-8 billion microbic bodies dense, in 1 ml according to the visual standard. The microbe suspension was given to the animals in warm milk after preliminary 24-hour starvation (1 ml of the microbe suspension to 2 ml of milk). The subsequent food ration of the infected kittens consisted of milk, cereal, bread, meat and water. They were fed three times a day.

EXPERIMENTAL RESULTS

During the beginning of the disease, we observed an increased temperature, the appearance of watery stool with pathologic foreign matter, and decline of general condition. In response to the infection, after the

incubation period all 16 kittens presented a clinical picture similar to the clinical picture of dysentery in humans. To confirm the diagnosis of dysentery, we used clinical observation, laboratory examinations, and we studied the pathologico-anatomical picture of the disease and the histo-morphological changes in the organs. In order to observe the development of the disease, the infected animals were sacrificed and autopsied at intervals ranging from the 6th to the 38th day after infection. The average incubation period was 3-6 days; the maximal period was 9 days, the minimal, 2 days.

All the experimental animals were divided into three groups according to the severity of the clinical course of the disease: 1) those with a serious form of dysentery, 2) those with dysentery of average seriousness, 3) those with a light form of the disease.

There were 3 kittens in the first group (No. 1, 8 and 14), one of which (No. 1) died on the 14th day after infection. The clinical picture of the disease in these animals was characterized by a violent beginning (marked increase in temperature, watery, bloody mucous stool, increased frequency of defecation, and marked decline in the animals' general condition—loss of appetite, sluggishness, intoxicated appearance—unsteady gait). Two days before the death of kitten No. 1, vomiting, convulsions and a condition of complete prostration were observed. In all of the kittens, tenesmus, anal distention and weakening of the sphincter were also noted. Bloody mucous material issued constantly from the anal orifice. In this group of animals, we traced the course of the illness up to autopsy (4-6-8 days). For the whole observation period, the kittens retained the increased temperature and pathologic stool, as well as the poor general condition.

The second group consisted of 5 kittens (No. 9, 10, 11, 13 and 16). These animals had a typical dysenteric affection, and their general condition was considerably disturbed. In all of the kittens, we observed a violent beginning period with a sharp increase in temperature, the appearance of frequent watery stool with pathologic foreign matter; in 3 kittens, blood and mucous were observed in the stool. The development of the disease was attended by tenesmus, anal distention, and weakening of the sphincter with involuntary defecation. These symptoms developed on a background of significant disturbance to the animal's general condition. The kittens lost their appetite and became sluggish as early as 1-2 days before becoming ill. On the 5th day of the disease, kitten No. 13 manifested an unsteady gait and convulsions.

The acute period of the illness lasted 3-6 days, and the high temperature continued for approximately the same period. In the kittens with a longer observation period (No. 11 and 16), the temperature fell below normal on the 6th-7th days of the disease and remained so until the end of observation. The watery stool remained during the entire observation period; blood was recorded in the stool during the 1st-4th days, mucous during the 2nd-9th days of the illness.

There were 8 kittens in the third group (those with a light form of dysentery). They all had a typical dysenteric affection without the symptoms of intoxication and with a relatively satisfactory general condition. As a rule, the majority of them increased in weight during the observation period.

In this group, the disease began with a slight or even normal temperature. In three of the kittens, the temperature only rose on the 5th-7th day of the disease. Frequent, watery stool appeared on the first day of sickness. Mucous was discovered in the stool of 5 kittens (see table). The kittens of this group were observed for a comparatively long period (up to 38 days), and it could be established that formed stools appeared on the 12th-16th days of the disease, although watery stools occurred periodically. The increased temperature was retained for 3-16 days, after which days of normal temperature alternated with slight exacerbations. One can also note that the longest incubation periods were most frequently recorded in the kittens of this group.

The clinical picture of the disease in all these three groups of animals indicated, first, that the disease appeared after an incubation period, second, that it was characterized by symptoms typical of dysenteric disease in man (especially in children), and third, that a definite pattern could be established in the development of the infection (acute period, followed by the gradual disappearance of the disease).

The development of clinical forms of the disease which varied as to gravity demonstrates the individual reactivity of the animals to the introduction of disease pathogens into the body.

We analyzed the possibility that the severity of the disease depends on the number of microbes which the animals received, or on the weight of the animal. We found that, in a given case, there was no relation between these factors. Thus, kitten No. 1, which had received 1 billion microbic bodies in 1 ml, had the serious form

of the disease, ending in death, while kitten No. 4, which had received 8 billions of microbes per 1 ml, was sick from a light form of dysentery. Both animals weighed approximately the same (kitten No. 1 – 510 g, kitten No. 4 – 640 g).

To confirm the clinical diagnosis of dysentery in the kittens, we conducted laboratory bacteriological examinations on the feces of the sick animals. The analyses were done daily from the 2nd day after infection until the end of observation. Bacteriological confirmation was received in all 16 kittens. Positive bacteriological cultures were recorded during the first 7 days of sickness. According to the frequency with which dysenteric bacilli were discovered, positive cultures were recorded $2^1/_2$ times more often in the first two groups (8 kittens) than in the last group (also 8 kittens), in spite of the fact that the observation period was longer in the last group. The more frequent cultures obtained of dysentery bacilli in the first two groups depended directly on the character of the stool, since the watery stools with blood and mucous gave 88% positive cultures, the watery stools with mucous, a little more than 50%, the watery stool without foreign matter, 26%, and the formed stool, a total of 2.4% positive cultures.

Bacteriological examinations were done on the internal organs of 15 of the animals after autopsy. For the examination, we used: blood from the heart, urine, bile, sections of the liver, spleen, mesenteric lymph nodes, kidneys and bone marrow, and also the contents and scrapings from the mucosa of the large and small intestines. The material was immediately cultured on favorable media. The dysenteric bacilli were isolated by the usual method.

Bacilli of Flexner's dysentery, type "W", were cultured from 15 kittens. Complete (100%) positive cultures were obtained from the contents of the rectum and from the mesenteric lymph nodes. In 13 (out of 15) kittens, pathogens were cultured from the spleen, in 11, from the contents and scrapings from the mucosa of the small intestine, in 7, from the contents and mucosa scrapings of the transverse colon, and in 5, from scrapings of the mucosa and the contents of the ascending colon. Dysentery bacilli were cultured from bone marrow in three kittens, and from the kidneys, in two.

To determine the immunobiological response of the body to the infection, we set up a reaction with the blood serum of the sick animals to obtain the level of agglutinin antibodies in the blood, and also specific and nonspecific phagocytic reactions to evaluate the change in cell reactivity.

The observations were made on 10 kittens. The production rate of agglutinin antibodies was determined in dynamics. The reaction was set up 3 to 6 times. Thirty-nine reactions in all were conducted. Each reaction was done with a live 18-hour culture of Flexner's type "W" No. 6176 in a dilution of $^1/_{3200}$ (see table).

The Dynamics of Agglutinin Production in the Blood Serum of the Infected Animals

No. of experiment	No. of kitten	Observation period (in days)	Number of reactions set up	Day after infection on which reaction was set up					
				3rd day	5th day	9th day	13th day	16th day	21st day
1	7	6	2	$^1/_{50}$	$^1/_{200}$	·	·	·	·
2	8	10	3	—	$^1/_{200}$	$^1/_{200}$	·	·	·
3	9	12	4	—	$^1/_{100}$	$^1/_{100}$	$^1/_{400}$	·	·
4	10	15	5	—	$^1/_{100}$	$^1/_{100}$	$^1/_{400}$	$^1/_{400}$	·
5	11	20	5	—	$^1/_{200}$	$^1/_{200}$	$^1/_{200}$	$^1/_{400}$	·
6	12	25	6	$^1/_{50}$	$^1/_{200}$	$^1/_{400}$	$^1/_{400}$	$^1/_{400}$	$^1/_{400}$
7	13	7	2	—	$^1/_{100}$	·	·	·	·
8	14	12	3	—	$^1/_{100}$	$^1/_{100}$	·	·	·
9	15	14	4	—	$^1/_{400}$	$^1/_{400}$	$^1/_{400}$	$^1/_{200}$	·
10	16		4	—	$^1/_{400}$	$^1/_{400}$	$^1/_{400}$	$^1/_{200}$	·

Explanation of symbols used: – ; negative reaction; · ; reaction not set up.

In analyzing the results obtained, one can note that a positive reaction appeared on the 6th day after infection, i.e., on the 1st-4th day of the sickness. On the 9th to 13th days after infection, the antibody titer

continued to increase (kittens No. 9, 10 and 11). In kitten No. 12, on the 15th day of sickness (the 21st day after infection) no decrease in the antibody titer could as yet be observed. The dynamics of the reactions in our experiment certainly reconfirm the development of a specific infection process in the animals. As for the comparatively slight elevation of the antibody titer (positive reaction in a dilution of $^1/_{400}$), analogous features have been noticed, as we know, in the results of the Widal test for dysentery in humans.

To further characterize the immunobiological response in the bodies of the infected animals, we conducted specific and nonspecific phagocytic reactions. To set up the reaction in the kittens, we took blood from the auricular vein in the morning on an empty stomach and mixed it with a 2.5% solution of sodium citrate in the following proportion: one part of citrate to one part of blood. To the blood citrate, we added one volume of an emulsion of a one-day culture of microbes, which had 1 billion microbes according to the visual standard. The mixture was put in an incubator for 30 minutes, after which thin smears were prepared. For the specific reaction, we used a strain of Flexner's "W" No. 6176 as the test microbes, and for the nonspecific, a culture of intestinal bacilli. As the activity index of the phagocytic reaction, a quantity of phagocytizing neutrophils, expressed in percentages was used. The intensity of phagocytosis was computed from the amount of bacilli in one microphage (arithmetic mean number). Observation was done on 10 kittens. We set up a series of reactions (on the 5th, 10th, 15th and 18th days after infection, and also before infection) in order to evaluate the phagocytic reaction in dynamics at different stages in the development of the infection. Even before infection, the indices of leukocyte activity were considerably lower in the specific reaction than in the nonspecific, which indicates the more active phagocytosis of the common pathogenic flora (the intestinal bacilli in particular).

When the dynamics of the phagocytic reaction was compared with the severity of the clinical picture of the disease in the first and second groups of animals (those with the more serious form of the disease), the indices of the activity and intensity of the leukocytes generally tended to decline sharply during the whole observation period both in the specific and in the nonspecific phagocytic reactions, which indicates the inhibition of both the specific and general reactivity of the body in the animals affected by dysentery.

The restoration of body reactivity proceeded more rapidly in the third group with the light form of dysentery, according to the indices of activity and intensity of the leukocytes to phagocytosis, and the index of phagocytosis intensity after the 10th post-infection day (4th-6th day of sickness) exceeded the original indices before infection (1.3-2.4).

According to the indices of accumulation in the blood of agglutinins and of change in cell reactivity, the immunobiological response of the body confirmed the development of a specific infection in the animals.

The dysentery diagnosis was also confirmed by the pathologico-anatomical picture of the disease. During the acute period of the disease, autopsy determined the following in the large intestine: hyperemia and edema of the mucosa, muco-purulent material, and punctate hemorrhages; in the small intestine: hyperemia, edema of the mucosa, and a small amount of muco-purulent material. During the second period of the disease (9th-18th days of sickness), the mucosa of the large and small intestines was pale, atrophic and its contour frequently blurred (especially in the small intestine). In the other internal organs, the following were observed during the acute period of the disease: the mesenteric lymph nodes were enlarged and dense; the liver was plethoric and bloody fluid oozed from a cut section; the spleen was more frequently enlarged and purplish-blue in color, with an uneven, granular surface. During the second half of the disease, areas of a clay-like color were observed in the liver, and the hepatic tissue was flaccid. The spleen remained enlarged and edematous and a sanious fluid oozed from a cut section; the kidney pattern was more frequently blurred.

The microscopic picture of the changes in the internal organs was studied in 12 kittens.

In the large intestine during the acute period of the disease were observed: desquamation of the surface epithelium, destruction of the connective tissue with the formation of microscopic ulcers, edema of the submucosa, changes in the vascular walls (especially in the submucosa) and the formation of a film on the mucosa with varying degrees of contact with the underlying tissue.

Along with degenerative processes, in the large intestine, by the 11th-12th day after infection, one could observe the regeneration of the mucous membrane, which was expressed in the growth of connective tissue replacing the destroyed mucosa. We did not observe the regeneration process to be completed even by the 25th day after infection.

The main microscopic observation in the mucosa of the small intestine was the destruction of its villi—considerable destruction to the actual membrane of the villi was observed as well as to their epithelium. This process began as early as the 6th day after infection, and reached its highest intensity by the 12th day. Other observations were: the infiltration of the mucous membrane, edema of the submucosa, dilatation of the mucosal vessels and deposits on the mucosal surface which were smaller than in the large intestine. Regeneration processes were observed in the small intestine as early as the 7th day after infection, although the regeneration of the mucous membrane had still not been completed by the 25th day after infection.

Microscopic observations in the liver were: dilatation of the portal vein capillaries and vacuolization of the hepatic cells, which was greater on the periphery of the lobule than in its center. In the red pulp of the spleen, an afflux of blood and a dark brown pigment were observed. In the same organ, the centers of multiplication of the white pulp were obscured, which could also be observed in the lymph nodes. In the cardiac muscle (especially during the second half of the disease), we found small foci of inflammation and the disintegration of the muscle fibers. In the kidneys, dilatation of the vessels could be seen, particularly on the boundary between the cortical and medullary substances. Some vacuolization of the tubular cells was also noted.

To conclude, one must remark that the profundity and character of the morphological changes do not depend on the severity of the disease; the time at which the animals were autopsied after the infection is the important thing here.

From the clinical and laboratory observations we made on the infected animals, we consider it possible to conclude that, in the conditions of the experiment, kittens manifest a typical dysentery and may serve as an experimental model for studying various problems of pathogenesis and immunity in dysentery.

SUMMARY

Experiments were carried out aimed to reproduce the experimental model of Flexner's dysentery in kittens.

All the kittens infected with the typical Flexner's strain showed after the incubation period (5 to 6 days) clinical symptoms similar to that of the dysentery in man.

Clinical and laboratory observations on the infected animals make it possible to assume that the kittens may serve as a model for studying problems of pathogenesis and immunity in bacillary dysentery.