THE PATHOGENESIS OF THE PULMONARY FORM OF EXPERIMENTAL TULAREMIA

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[Following is the translation of an article by R. A. Savelyeva and Ye. V. Ananova, Gamaleya Institute of Epidemiology and Microbiology, AMN, USSR, published in the Russian-language periodical Zhurnal Mikrobiologii Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology) No 3, 1965, pages 65--70. It was submitted on 18 Nov 1963. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

In 1934 Rudnev, and also Volferts, distinguished primary pulmonary tularemia as an independent clinical form of disease, originating by aspiration infection. Subsequently this clinical form was studied by Rudnev and others and it received experimental confirmation in the works by Linnik and Khakhina.

The mission of this work includes the further experimental study of infection by the aspiration method and also clearing up the peculiarities of the pathogenesis of the pulmonary form of tularemia.

The highly virulent tularemia microbe strain No 503 was used in the observations.

In the first series of tests * on guinea pigs and white mice, we attempted to ascertain the minimum infecting doses in the aspiration method of administering tularemia bacteria.

* Taking part in the setting up of these tests was a scientific co-worker of the tularemia laboratory, G. P. Uglovoy.

The animals were infected in a special chamber with a capacity of about 1 m^3, where the bacterial suspension was sprayed in the form of a very fine mist from two devices. Usually 20--40 ml of bacterial suspension was consumed in the course of an hour. For determining the number of bacteria which were found in a suspended state in the chamber, at the beginning and at the end of the operation we took samples of the air with the help of a Krotov device. Ten ml of physiological solution was poured into a Petri dish, the dish was placed in the device referred to, and 100 liters of air from the chamber was passed through the liquid. The resulting suspension was then titrated on white mice (they were infected with reduced doses), and based on their death the number of microbial cells in 1 ml of liquid was determined, and consequently in 10 liters of air from the chamber.

By means of numerous verifications it was established that when spraying a suspension containing 100 microbial cells in 1 ml (based on the optical standard), in 100 liters of air from the chamber there were around 10 bacteria, when spraying 10,000 microbial cells -- around 1,000 bacteria, etc. Samples taken in 15 and 45 minutes following the onset of operation of the chamber did not essentially differ based on the number of microbes. In addition to this, we determined the number of microbes in the suspension being sprayed prior to switching on the devices and following completion of the spraying. If the number of bacteria was lowered in comparison with the initial number, we did not consider the result of the test.

The animals were placed in the chamber freely in small groups. In order to keep them from licking their wool, little collars were placed on their necks. The pigs' eyes were patched up in order to prevent the possibility of infection through the mucous membrane. After infection the patches and collars were removed from the animals and the wool was treated with alcohol. The actual number of bacteria entering the lungs of the animals, that is the infection dose, was calculated on the basis of the number of bacteria in 100 liters of air (depending on the concentration of the suspension being sprayed), the minute volume of the lungs with a calculation of animal weight, and the time of exposure in the chamber. It is understood that the calculated infection doses had only an approximate significance, however, they turned out to be quite close to actual. For example, in two guinea pigs immediately following infection by the aspiration method with 3,000--4,000 microbial cells (by a 30-minute spraying of a suspension containing 1 million microbial cells in 1 ml), 3,000 bacteria were detected in the lungs, and following infection with 6,000--8,000 microbial cells -- 4,500 bacteria. The latter was determined by the method of titrating pulmonary tissue on white mice.**

** The method of titration is described in the book "Tularemia" -- edited by N. G. Olsufyev and G. P. Rudnev, page 98.

Used all told in the tests were 35 guinea pigs weighing 350--400 grams and 37 white mice weighing 12--15 grams. When setting up the tests on the guinea pigs, white mice were placed in the chamber at the same time with them. Three tests were set up only on mice (see table). In summing up, it developed that when single bacteria (6--8 specimens) ended up in the guinea pigs' respiratory tract, infection set in along with the death of a large portion or all of the animals used in the test. The white mice also showed a high susceptibility and sensitivity to infection by the aspiration route -- 5 microbial cells caused the death from tularemia of all the experimental animals. It was noted that with the presence of 100 bacteria in 100 liters of air, the majority of pigs were infected and only individual mice (tests No 2, 3, 4). This is explained by the fact that in guinea pigs the lungs have a large respiratory capacity and they can aspire approximately 6--8 times more bacteria than mice.

Thus, the infection of guinea pigs and white mice by the aspiration route set in following the introduction of almost the same minimum doses of tularemia bacteria as during the subcutaneous or intracutaneous infection.

In all the cases of the death of animals following aspiration infection, besides the changes in the spleen, liver, and other organs which are typical for tularemia, expressed inflammatory changes were detected in the lungs -- either a sharp hyperemia of separate lobes or the entire organ (white mice), or massive sectors of hepatization and numerous necrotic nodules (guinea pigs).

Subsequently it was interesting to ascertain the sensitivity of white rats to aspiration infection. As is known, they belong to the second group. The method of infection and the strain of tularemia microbe were the same as in the tests on guinea pigs and mice. Following administration of 5,000--6,000 microbial cells, 4 out of 6 rats died from tularemia on the 6--14th day, and 50,000--60,000 aspired bacteria turned out to be a completely lethal dose for these animals. They died on the 5--9th day with a pathologoanatomical picture which was characteristic for tularemia infection and significant lesions in the lungs. Following the administration of 500--600 microbial cells, all the animals survived. Thus, the white rats proved to be considerably more sensitive to aspiration infection than to subcutaneous, during which a fully lethal dose for white rats comprised 100 million or one billion microbial cells.

In the second series of tests, which were set up on guinea pigs, we followed the accumulation of tularemia bacteria and the pathomorphological changes in the pulmonary tissue throughout the course of the infection process following aspiration infection. The tests were set up twice.

Twenty-two guinea pigs were subjected to infection in the chamber. The conditions for infection were as follows: Exposure in the chamber -- one hour, suspension concentration -- one million microbial cells in 1 ml. Immediately after infection, and also in 1, 2, 3, and 4 days two pigs each were destroyed and their organs investigated. A suspension of organs was introduced subcutaneously to white mice in order to establish the distribution of tularemia bacteria in the organs, and a suspension of organs was inoculated on convolute vitelline medium (figure 1). For the purpose of determining the accumulation of bacteria in the lungs, pulmonary tissue was titrated on white mice. The organs of guinea pigs were also subjected to pathomorphological investigation. Material for pathohistological investigation (lungs, tracheobronchial lymph nodes, spleen, liver) was fixed in 10% neutral formalin, and sections were stained with hematoxylin-eosin. Organs from healthy, non-infected animals and those which died from the stated infection (4--6th day) were subjected to analogous investigations. During autopsy immediately after infection, visible pathologoanatomical changes were not exposed. Tularemia causative agents were detected only in the trachea and lungs, including in their most outlying sections. In

one gram of pulmonary tissue 1,000 microbial cells were detected. Upon histological investigation of the pulmonary tissue of infected animals, in contrast to the noninfected (control), polymorphocellular infiltrates with a large number of lymphoid elements were detected perivascularly and peribronchially. These infiltrates were unique "sockets" (figure 2 on the inset). The tracheobronchial lymph nodes, and also the spleen and liver, preserved their usual structure.

After 24 hours a febrile reaction was noted in all the animals. Upon autopsy a uniform moderate hyperemia of the lungs and the tracheobronchial lymph nodes was detected and the dimensions of the latter were not increased. There were no changes in the other internal organs. The causative agent was detected in the trachea, lungs, tracheobronchial lymph nodes, spleen, and blood, which indicated a generalization of the process. In one gram of pulmonary tissue up to 100,000 microbial cells were detected. Upon histological investigation there was noted in the pulmonary tissue a swelling of the majority of the interalveolar septa, on the background of which occurred the formation of polymorphocellular nodules, reminescent of tularemia granulomae. The structure of the tracheobronchial lymph nodes was completely preserved. Against a background of plethora of the organ there was hyperplasia of reticular elements and insignificant hypertrophy of the secondary nocules. Analogous symptoms were detected in the spleen.

After two days a uniform hyperemia of the lungs was noted, along with an insignificant enlargement and plethora of the spleen and liver. The tularemia causative agent was detected in all the organs; one gram of lungs contained up to one million microbial cells. Histologically, a sharp expansion and plethora of the vessels were detected in the lungs. In addition to the earlier described pathohistological changes, the following were established in the pulmonary tissue. There were vast sections, devoid of alveolar structure and representing solid pneumonic foci, among which there were distinct typical tularemia granulomae with symptoms of necrobiosis in the center (figure 3 in the inset). In the tracheobronchial nodes the structure was preserved, but from time to time in them it was possible to see cellular accumulations in the form of nodules, and individual cells of these nodules were found in a condition of necrobiosis. In the spleen there was hypertrophy of the Malpighian bodies, hyperplasia of the red pulp, and small cellular accumulations of a nodular nature. In individual nodule-granulomae the cells were found in a state of necrobiosis and necrosis.

In pigs which were destroyed after three days there was an enlargement, induration, and hyperemia of the tracheobronchial lymph nodes, diffuse hyperemia and small foci of hepatization in the lungs, and on the surface -- numerous minute gray nodules, enlargement and induration of the spleen and liver. The tularemia microbe was detected in all the organs, and in the lungs -- up to 10 million bacteria for one gram of tissue. Upon histological investigation it was established that almost all the tissue of

the organ had changed pathologically: An expressed plethora of the organ with a picture of stasis in individual vessels, a large number of granulomae of a polymorphocellular composition with necrosis in the center; some extensive granulomae with several centers of necrotization were formed as a result of the fusion of more minute individual granulomae. All the tissue of the tracheobronchial lymph node, and also the spleen and liver, were affected with a granulomatous process which is specific for tularemia.

After four days, when the death of individual pigs was already observed, and in later periods all the characteristic pathologoanatomical changes for tularemia were noted. One gram of lung contained 100 million -- 1 billion microbial cells. Histologically, vast foci of specific pneumonia with symptoms of necrobiosis and necrosis were detected in the lungs, along with focal-purulent bronchiolitis and the symptoms of catarrhal-purulent bronchitis. The structure of the regional lymph nodes was completely disrupted. Only in places the lymphoid tissue was preserved in the form of islets. A great number of granulomae running together is apparent. There is an analogous picture in the spleen.

The data obtained permits the assumption that in the first hours after aspiration infection with a finely dispersed suspension the tularemia microbe penetrates directly into the pulmonary tissue, the infectious process begins from it and develops similar to a type of primary pneumonia. Having penetrated into the peribronchial spaces, the microbe and the products of its disruption produce the symptoms of peribronchial and perivascular infiltration with the subsequent transition into the wall of the bronchus. The dose and the method of infection used by us caused a relatively rapid penetration of the microbes into the blood channel, therefore, the local process in the lungs to a certain degree was also the result of hematogenous dissemination. The death of the animals was caused by the respiratory surface of the lungs being put out of service and the manifestations of a general profound toxicosis. It is understood that during aspiration infection some of the microbes would also reach the mucosa of the trachea and the bronchi, but here, apparently, the conditions are less favorable for survival and multiplication.

Our results correspond with the results of Lobanov et al. (1960), who studied the pathogenesis of plague infection following aspiration infection, and the experimental investigations of Kolesnik and Ekakhina on the pulmonary form of tularemia. Our material and the materials from these authors show that during the aspiration method of infection the development of specific inflammatory foci in the lungs outstrips the development of pathological changes in the other organs, including in the tracheobronchial lymph nodes.

Conclusions

1. Guinea pigs and white mice are susceptible and sensitive to tularemia under the conditions of the aspiration method of infection almost to the same degree as during subcutaneous infection, while white rats turned out to be more sensitive.

2. In an experiment on guinea pigs the feasibility was confirmed of the development of primary tularemia pneumonia. The inflammatory process begins with the pulmonary tissue, is spread further to the tracheobronchial lymph nodes and concludes with the subsequent rapid generalization of the infection.

3. The specific inflammatory process in the lungs of guinea pigs is made up from a number of successive phases: Partial atelectasis, plethora and edema of the interalveolar septa with the formation of polymorphocellular infiltrates; further it acquired a specific granulomatous nature with the symptoms of necrobiosis and necrosis.

Literature

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Susceptibility and sensitivity of guinea pigs and white mice to the tularemia microbe under the conditions of aspiration infection.

No. of test	Concentration of microbes in 1 ml of sprayed suspension	Number of bacteria in 100 liters of air	Exposure (in min.)	Guinea pigs			White mice		
				Probable number of aspired microbial cells *	Result of infection	Period of death of animals (in days)	Probable number of aspired microbial cells**	Result of infection	Period of death of animals (in days)
1	10	1	60	0	0/5	--	0	0/5	--
2	100	10	60	<1	0/5	--	0	0/5	--
3	1000	100	60	6--8	3/5	10--12	1	0/4	--
4	1000	100	60	6--8	4/5	9--12	1	0/4	--
5	1000	100	60	6--8	5/5	10--13	1	3/5	9--12
6	10 000	1000	30	Tests not set up			5	5/5	9
7	1 million	100 000	30				500	4/4	6--7
8	100 million	10 million	30				50 000	5/5	6

* Minute volume of guinea pig lungs accepted as 0.35 ml for 1 gram of weight.
** Minute volume of white mouse lungs accepted as 1.1 ml for 1 gram of weight.
Designations: Numerator -- number of animals died from tularemia, denominator -- total number of animals used in test.

Period of investigation

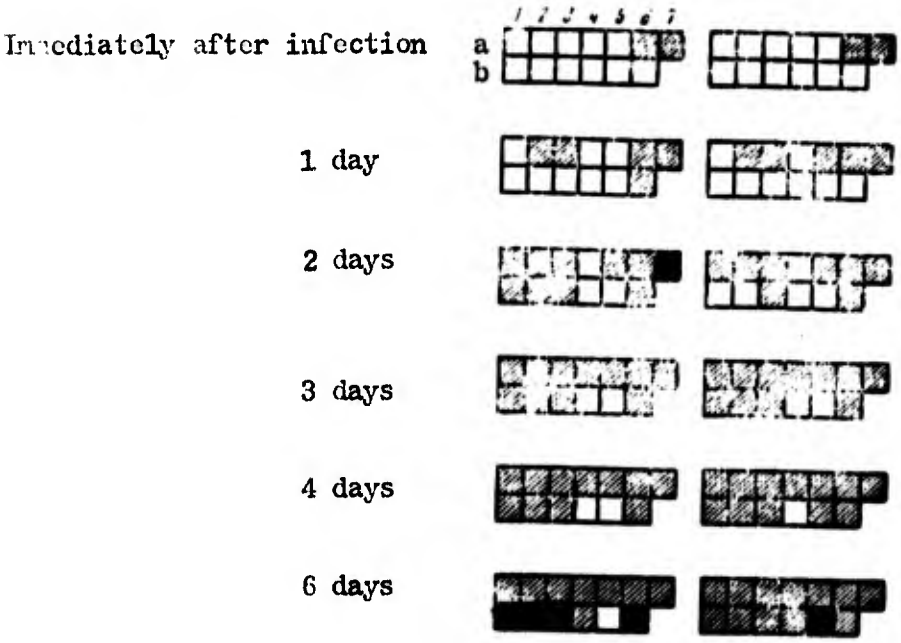

Figure 1. Results of the biological and bacteriological investigation of the organs and tissues of guinea pigs in various periods after aspiration infection with the virulent strain No 503 of the tularemia microbe. Each figure designates one animal.

a - biological tests (on white mice), b - seedings on vitelline medium.

1 -- cervical lymph node; 2 - tracheobronchial lymph node; 3 - spleen; 4 - bone marrow; 5 - blood; 6 - lungs; 7 - trachea. Positive results of investigation are crosshatched with stripes, the solid black represents an extraneous infection.

Figure 2. Pulmonary tissue of a guinea pig destroyed immediately after infection. The pulmonary tissue is mainly pneumatic. There are polymorphocellular accumulations around the bronchi and vessels. Magnification 126 x.

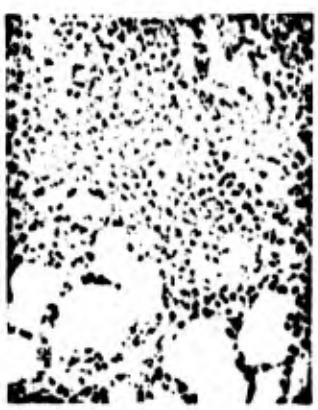

Figure 3. Pulmonary tissue of a guinea pig destroyed in two days following infection. There is extensive tularemia granuloma, the individual cells of which are found in a state of necrobiosis. Magnification 126 x.