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On the question of the diagnostic significance of biological reactions and chemotherapy in human glanders.

by S. S. Sabolotny

From the laboratory of the Clinic for Infectious Diseases of Simferopol University. Centralbl. f. Bakt. 1 Abt. Orig. Vol. 97, No. 2/3, pp 168-173, 1926.

I.

I. In the case of many infectious diseases the bacteriological diagnosis is made nowadays not only through discovery of the antigen, the pathogen, but also by the determination of the specific antibody in the serum of the diseased organism — serodiagnosis — and by the presence of a hypersensitivity to the suspected antigen.

The diagnosis of a glanders infection in veterinary practice is based almost exclusively on the method of antibody-determination, including that of anaphylaxis; in this connection the reactions of complement fixation and hypersensitivity to mallein have proved to be especially successful.

Now it is precisely these biological reactions that have not been exhaustively examined in the human. There are few items in the literature, and the authors limit themselves to the assumption of an analog between the organisms of man and horse. Consequently one must resort to a complicated method of isolating pure cultures and to animal experiments for the purpose of diagnosis, all of which is very inconvenient. It requires at least 10-14 days (if the test is made with guinea pigs) and is not without danger, besides. This is the reason why we allow ourselves to report the results of biological reaction tests obtained in 2 cases of human glanders, diagnosed by Koch's method.

First we shall list some data from the anamnesis.

1. Er-r., German colonist, 22 years, admitted to the clinic on 2 May 1923. The patient is normally built and nourished. Approximately 1½ months ago rheumatic pains in the extremities appeared, which could not be removed by antirheumatic measures. During the last 3 weeks the condition of the patient has deteriorated; he complained of pain in the left side. On the day of admission: Lightly fluctuating infiltrate on the dorsal side of the right foot and a small, painful infiltrate at the left shoulder; in addition, severe pain at the right shoulder without objective symptoms; temp. 38.0°C. Blood sterile. From the pus of the infiltrate at the right foot we obtained a culture of B. mallei (4 May). The same pus, introduced into the abdominal cavity of the guinea pig (male), evoked the typical glanders process and after sowing parts of the animal's organs I obtained a pure glanders culture. (During inspecti of the patient's farm by a veterinarian, horses with glanders were found).

Leukocytic formula (17 May): Lymphocytes 21%, neutrophils 79%, mononuclear cells and transitional forms 0.6%, eosinophils 0%, no pathological forms. Erythrocytes did not show abnormal forms. Urine normal.

The condition of the patient during the first 2-3 weeks in the clinic was satisfactory, despite the elevated temperature. However, later the temperature which previously had fluctuated between 37° and 38.5°C, rose to 40°C, his strength began to ebb; new, very painful infiltrates appeared, his appetite decreased. On 9 June the patient died. Both agglutination and precipitation methods were used in connection with this patient (10 May) and Salvarsan was injected (17 May).

2. Chw-ow, 19 years, laborer (had worked in a village), was admitted to the clinic in the first days of the illness on 8 January 1924 with a high temperature. He complained of pains primarily in the extremities. The patient is poorly nourished, pale, anemic, his face is earthen, no local symptoms. After a few days pain and tumescence appeared at the left shoulder and the left shoulder blade, subsequently also in the right shoulder.

Biopsies were performed, but micro-organisms could not be demonstrated either in slide preparations (Loffler, Gram, Ziehl-Neelsen) or in cultures. Later, temporary new subcutaneous infiltrates appeared, primarily in the limbs and the face. Unless they occurred in the areas of the joints, the patient was bothered relatively little by them.

At the beginning of March glanders was being suspected, but repeated sowing of pus and blood failed to produce positive results.

We only succeeded toward the end of April in obtaining a culture from an infiltrate which actually proved to be a glanders culture (growth on the media and tests with guinea pigs and mice).

Since the admission of the patient approximately one year had passed. Despite the relatively severe infection, despite the high temperature of a type at times remittent, at times intermittent with rises up to 39-40°C and the presence of occasionally very painful nodules in the skin and in the deeper tissual strata, the patient bore his affliction well and complained rarely, although he looked quite exhausted. He took to his bed only when the joints were strongly attacked; at the slightest improvement he left his room and walked in the yard. Recently his overall condition has deteriorated to such an extent that he can no longer get up and cannot even take his meals without assistance.

Hemal analysis (29 May): In 1 ccm erythrocytes 3, 700, 000, white blood cells 6,300, hemoglobin 65%, stain index 0.9. In stained smears the erythrocytes do not deviate from the normal. Leukocytic formula: Lymphocytes 47%, neutrophils 46%, eosinophils 2.3%, mononuclear cells and transitional forms 5%, no pathological forms; concerning Bizzozero's platelet, no apparent deviations; no parasites. This patient was subjected to tests for the following reactions: Agglutination 5 March, 19 April and 10 September; precipitation 29 May, and complement fixation 25 May; he was treated with mallein twice (8 July and 11 September); the agglutination test on 10 September was conducted 2 months after the first mallein test; the interval was sufficiently long to avoid the possibility of an artificial appearance of antibody under the sole effect of mallein.

Therapeutically the patient received neosalvarsan (31 May, 10 June, and 26 June) and the preparation "Bayer 205" (30 October, 1 November and 13 Nov.).

In proceeding to the discussion of the tests conducted by us, we should like, for the sake of clearer perspective, to summarize all related tests in one general tabulation. In order to evaluate the results more precisely, each test was simultaneously performed with normal sera, which at all events did not come from persons with glanders. A reliable glanders organism was utilized in the form of rabbit No. 2, in which an inactivated culture of B. mallei was instilled ad hoc, since a more suitable material was not available.

A. The agglutinizing effect of serum in humans with glanders was first observed by Dedjulin (1). Race (2) and Desson (3) point out that even normal human serum agglutinates glanders bacilli in high dilutions — according to Metsch (4) 1/500 —, therefore only higher dilutions — according to Hildemeister and Jahn (5) 1/800 — can be considered specific. In our cases the test was performed macroscopically. We used an active, 30-48 hour agar culture of B. mallei as antigen. We also had 16 normal control sera: 10 from clinically healthy individuals which in one half of the cases showed a clear agglutination at dilutions of 1/50 and 1/100, and 6 sera from patients (t. abd., cholelithiasis, tetanus, pyemia), which only showed an indistinct agglutination, and this at a dilution of not higher than 1/25. The agglutination results with serum of our glanders patients are listed in table I.

Table I *)

| Name of patient | | Serum dilution | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1/50 | 1/100 | 1/200 | 1/400 | 1/500 | 1/800 | 1/1000 | Control |
| 1.E-r**) | 10 May 1923 | 3/ | 3/ | 3/ | 3/ | 2/ | 2/ | 2/ | - |
| 2.Ch-w | 5 Mar 1924 | - | - | - | - | - | - | - | - |
| | 19 Apr 1924 | 2/ | 2/ | 2/ | / | / | - | - | - |
| | 10 Sep 1924 | 2/ | 2/ | - | - | - | - | - | - |
| 3.Rabbit No. 2 | | 3/ | 3/ | 3/ | 3/ | 3/ | 2/ | / | - |

Footnotes: *) Designations of the results according to the scale: 3/ equals complete agglutination, 1/ equals distinctly visible agglutination viewed through the magnifying glass. The positive result, in the case of weaker dilutions, was obtained already after a 2-hour storage of the tube in the thermostat; it was more distinct, however, and that also at higher dilutions, after 12-24 hours at room temperature. **) Unfortunately the complete titer was not determined in the case of this patient.

As is evident from this table, strong fluctuations occurred in the agglutination titer in the case of Chw-ow, who was tested 3 times (the test was conducted with 2 completely agglutinable strains).

We meet here in the human the inconsistencies in the agglutination titer well known in veterinary practice of equine glanders.

B. The precipitation test (we have no data on literature) was performed according to the "substrate" method, in which only the antigen (the Russian liquid mallein from the veterinary institute at Charkow) is titrated, while the tested sera remain undiluted. Results are presented in table II.

Table II

| Name of patient | Antigen dilution undiluted | 1/5 | 1/10 |
|---|---|---|---|
| 1. E-r 10 May 1923 | + | · | · |
| 2. Ch-w 29 May 1924 | + | + | · |
| 3. Rabbit No. 2 | + | + | + |

The reaction was fast and already took place during the commencement of the test; it became especially distinct following storage for 30 minutes in the thermostat. Neither antigen nor sera per se showed turbidness with physiological sodium chloride.

The 2 normal human sera and the normal rabbit serum used as control gave negative results. The serum of rabbit No. 2 revealed a more distinct ring.

C. Complement fixing antibody was found by P. Maslokovetz in Professor Wyschelessky (6) who had glanders and recovered, and also by Dedjulin in the case described by Dr. Jurtschenko (9). Gildemeister and Jahn (5) in their cases of human glanders found antibody up to a very high titer of 0.01. Our tests were conducted with the sera of the patient Chw-ow, rabbit No. 2 and with 2 sera (without glanders) of the patient L-w (t. exant.) and a healthy woman, Mrs. S., in which the inhibitory and hemolytic properties of the sera and the antigen were examined.

Table III

| Name of patient | Serum dosis 0.2 | 0.1 | 0.05 | 0.025 |
|---|---|---|---|---|
| 1. Ch-w | 4+ | 4+ | 4+ | 2+ |
| 2. Rabbit No. 2 | 4+ | 4+ | 4+ | 3+ |

The inactivated (60°C - 2 hours) emulsion of a glanders agar culture served as antigen. The amboceptor was used in a threefold dosis. First the antigen was titrated with respect to each of the sera in doses of 0.2. The titer was 0.1. After we had thus ascertained the presence of Bordet's antibody in the first two sera, we began to titrate them with respect to the determined dosis of antigen (0.1). The result is presented in table III. Complete fixation is represented by 4+.

As the table shows, the titer of the two "glanders-containing" sera is 0.05, again slightly higher in the rabbit than in Chw-ow.

D. Data contained in the literature concerning malleinization do not agree. Besson (3) considers the subcutaneous method of malleinization as dangerous to humans, and, like Wladimirow (7), he recommends the skin test after Martel, which however had a negative result in one of Prof. Flerow's cases (8). Hetsch (4) on the other hand, on the basis of the observations by Babes, Bonome, Zieler and Buschke, considers subcutaneous malleinization of humans to be quite safe and recommends it strongly.

In our case the patient Chw-ow was subjected to malleinization twice, with an interval of 2 months -- on 8 July on the back and on 11 September on the abdomen. The aforementioned mallein served as antigen; injection was accomplished at 12 o'clock midnight. A control *) was furnished by A-k and B-ow, who had just recovered from malaria. The temperature of Chw-ow, A-k and B-ow was taken before and after malleinization every 3 hours for 4-5 days. A-k had weak pains upon pressure at the site of injection, unimportant and short-lived pains in the abdomen and diarrhea with a single temperature depression to 35.8°C, which can be explained by a dietary error, while B-ow only complained of "discomfort" on the next day.

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Footnote: *) It is interesting to note that rabbit No. 2, which was immunized with an inactivated culture of B. mallei and easily produced the above antibody, just as the fresh rabbit, did not react to mallein at all. It seems that mere dead bacterial bodies are insufficient for production of anaphylactic antibody and that the products of their vital activity are necessary.

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Concerning the patient Chw-ow, the reaction was as follows the first time: In contrast to the 2 days preceding and following, the temperature 12-15 hours after injection rose by 1°C; a headache not previously observed occurred, and the pains in the involved knee joint increased. At the site of injection (the back) a small, painful, not reddened swelling was seen, which hindered body movements.

After second malleinization the following occurred: Headache, pains in the involved left knee joint without swelling, and a definitely positive local reaction on the abdomen, which manifested itself in a significant thickening of the abdominal wall on the injected side (without swelling), in redness and severe painfulness; in the corresponding inguinal region, where previously a collection of glands had been present, one of the glands was very painful under pressure. The exudate from the wounds seemed to increase; the temperature during the first 24 hours remained the same as on previous days, i.e. it fluctuated between 37 and 39°C; on the following day, however, in connection with profuse perspiration unusual in this patient, the temperature sank to 35.5-38°C. Sweating was repeated in the next days with corresponding depressions in temp.

At the end of the first part of our report we should like to add the following pronouncements.

1. Strictly specific antibodies are produced in the organism of humans with glanders: agglutinins, precipitins, complement-fixing and anaphylactic bodies; the diagnosis of glanders can be made according to their presence. 2. The agglutination titer, at least in cases of chronic glanders, are subject to strong fluctuations up to complete disappearance in the same patient. ~~(see patient Chw-ow)~~. This fact is important to the extent that negative results of a single test may not be construed to exclude glanders. 3. The presence of normal agglutinins in humans does not represent a hindrance to the interpretation of test results. 4. Malleinization, even subcutaneously, is quite safe for humans and offers valuable diagnostic intelligence.

II. We now come to chemotherapeutic observations:

The experiments of Beniwolensky (1) with Salvarsan which showed that this preparation kills glanders cultures in vitro in 1 minute at a dilution of 1/40,000, at 1/100,000 in 3 minutes, at 1/1,000,000 in 15 minutes, have stimulated us to test this preparation in vivo in humans, although according to Gorjaew and Blagodetelew (1) it only causes improvement in horses and according to Miessner had no effect at all.

We have used salvarsan intravenously in 3 cases of human glanders: In the above 2 cases and in a third whose diagnosis was made solely by Koch's method, without serologic tests. In the following presentation we shall use a chronologic sequence.

No. 1. 6 March 1922. Severe acute glanders in a 10 year old boy with glanders bacteriemia. On 8 March 0.3 Novarsenobenzol Billons was dispensed without any effect. The patient died on the next day.

No. 2. 2 May 1923. Patient Er-r (see above). After instillation of 0.9 Novarsenobenzol Billons on 17 May a short reaction occurred (chills, rise in temperature, diarrhea). No effect on the overall course of the disease was noted. The symptoms became more marked and the patient died on 9 June.

No. 3. 8 January 1924. Patient Chw-ow (see above). A case of torpid glanders. Neosalvarsan dispensed on 31 May, 0.6; on 10 June, 0.5; and on 26 June, 0.9; only a strong depression in the temperature from 39° to 35.5°C with copious perspiration could be noted on the second day after the first injection. The temperature was normal in the next 3-4 days and then relapsed into the irregular type of fever characteristic for the patient. Later, in the interval between the first and second instillations the superficial wounds began to heal, the deeper ones, strongly purulent, remained unchanged. Moreover, soon after the first injection new infiltrates occurred. Subsequent dispensations of Preparation "914" had no effect whatever. In Dr. Jurtschenko's case (9) treatment with salvarsan also remained without effect. We meet here in connection with B. mallei the paradoxical fact long known in chemotherapy, that a preparation which proved effective in vitro shows itself inactive in vivo — a fact easily explained: The organotropy of the preparation is stronger than its parasitotropy. — Our experimental data concerning the utilization of the trypanocidal preparation "Bayer 205" are much more limited: Prof. W. Yakimow, to whom we owe gratitude for the valuable data concerning the preparation and the preparation itself, has demonstrated in his tests that it does not kill B. mallei in vitro (verbal communication). Nevertheless, based on the fact also known in chemotherapy that a preparation inactive in vitro may show effect in vivo, we decided to use it on a human being (the patient Chw-ow), especially since this preparation had never been tested on a living organism with glanders. The preparation was instilled 3 times intravenously, a total of 1.0: On 30 Oct. 0.25, on 1 Nov. 0.25, and on 13 Nov. 0.5. Part of the preparation which got underneath the skin, caused a painful swelling and redness which lasted several days. The previously tested urine showed only a low specific gravity. After each injection, especially after the two first ones, the following reaction was noted. Rise in temperature, pains in the head and over the entire body and especially in the wounds, nausea and weakness; no changes occurred in the

kidneys and intestines. The therapeutic effect was nil. The rubbing in of 2.0 unguentum cinereum pro die during December also had no effect, a treatment recommended in glanders which was used on Prof, Myschelessky (6) who was cured of the disease.

Salvarsan as well as "205 Bayer" proved to be completely ineffective in our cases of glanders.

After completion of our paper, the patient Chw-ow died on 15 January 1925 with symptoms of increasing cardiac weakness, after spending 1 year and 1 week in the clinic. During section many glanders nodules were found in the spleen, the liver and especially in the lungs.